

## Synthetic Gallic Acid Derivatives as Models for a Comprehensive Study of Antioxidant Activity

by Florence Belin, Philippe Barthélémy\*, Karine Ruiz, Jean Michel Lacombe, and Bernard Pucci\*

Université d'Avignon, Laboratoire de Chimie Bioorganique et des Systèmes Moléculaires Vectoriels,  
Faculté des Sciences, 33 rue Louis Pasteur, F-84000 Avignon

(tel.: 33(0)490144442; fax: 33(0)490144449; e-mails: bernard.pucci@univ-avignon.fr,  
philippe.barthelemy@univ-avignon.fr)

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The synthesis and antioxidant efficiencies of amphiphilic gallic acid derivatives are reported. To specify the impact of chemical structure on the antioxidant efficiency, several structural modifications of gallic acid were performed. The following structural features were chosen: *i*) introduction of hydrophobic or hydrophilic residues on the gallic acid and the type of their linkage, *ii*) the hydrophilic and/or lipophilic character of the whole molecule. The physico-chemical studies of the different series prepared revealed that the antioxidant efficiency of this polyphenol depends clearly on the nature of the linkage with both hydrophilic and hydrophobic parts. A push-pull effect is always necessary, and ester or amide bonds seem well adapted to increase the antioxidant efficiency. Second, under the oxidation conditions applied, it was observed that the hydrophilic and/or lipophilic character affects drastically the antioxidant activity of gallic acid derivatives. The results obtained are in accordance with the polar paradox, hydrophobic derivatives inhibit oxidation in an aqueous phase, whereas hydrophilic products are not efficient.

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**Introduction.** – It is known that diets rich in vegetable and/or fruits may induce a frequency decrease of cardiovascular diseases or certain cancers. Among numerous constituents found in these foodstuffs, antioxidants such as vitamins, carotenoids, sterols, and polyphenols are assumed to be responsible for the protective effects observed [1]. Natural and artificial antioxidants in human food allow stabilization of the quality of products by inhibiting free-radical formation and photosensitized oxidation processes. Indeed, in several cases, these oxidative phenomena can lead to toxic derivatives and, therefore, affect the safety of lipid in food [2].

Several works [3] have been reported in the literature on the effectiveness of antioxidants with different substrates, but also on systems, oxidation conditions, and methods to evaluate lipid autoxidation. However, such a diversity has led to confusing and sometimes to conflicting results. One can note that it is quite difficult to properly determine the activity of antioxidant derivatives in oils and/or food emulsions due to their complex interfacial affinities between air-oil and oil-water interfaces [4]. Consequently, the methodology and the nature of antioxidant derivatives (purity, extract, hydrophilic and/or lipophilic character<sup>1</sup>) must be carefully considered to avoid nonvalid interpretations and/or conclusions. Indeed, the antioxidative activity of a

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<sup>1</sup>) *Griffin* was the first to suggest an empirical scale he called hydrophilic–lipophilic balance (HLB). Basically, surfactants with low values of HLB are more compatible with the oil phase (are more hydrophobic) and tend to stabilize the H<sub>2</sub>O in oil (W/O) emulsions, while those with high HLB values are more compatible with H<sub>2</sub>O (are more hydrophilic) and tend to stabilize the O/W emulsions [15].

given compound depends upon numerous parameters with respect to its structure and physico-chemical properties, but also on the reaction conditions used. As a result, to rationalize the relations between the structure and antioxidant efficiency in controlled media, it seems nowadays necessary to develop new synthetic models with adjustable structures derived from natural antioxidant compounds.

The aim of this work is to synthesize new antioxidative molecules derived from gallic acid (= 3,4,5-trihydroxybenzoic acid). The antioxidant properties of polyphenols are well-known. In addition, methyl, propyl, and/or lauryl gallates are widely used as food additives because of their antioxidant properties. Thus, this polyphenolic structure is very often found in the diet. Structural modifications of gallic acid were undertaken in order to specify the role of the following variable parameters: *i*) the nature of the linkage to both hydrophobic and hydrophilic residue, *ii*) the amphiphilic character of the whole molecule, *iii*) the number of galloyl units grafted on a given backbone. Then, the impact of these structural variations on the antioxidant activity was evaluated by using a procedure adapted from the literature. This test was achieved in an oil-in-water (O/W) emulsion made of  $\beta$ -carotene, linoleic acid,  $H_2O$ , and a nonionic surfactant [6]. These conditions could be compared to the physiological environment of the natural antioxidant.

**Antioxidant Activity.** – In the case of O/W emulsions, the tests based on the co-oxidation of  $\beta$ -carotene are very often used in the literature because this method is simple and sensitive [7][8]. This method was described first by *Marco* [9], then modified by *Miller* [10] and *Pratt* [11][12]. Recently, the  $\beta$ -carotene bleaching method was used to rapidly test samples for potential antioxidant activity [7].

We chose to adapt the procedure reported by *Chevolleau et al.* [6] to our molecules. In this test, the  $\beta$ -carotene is co-oxidized by linoleic acid in the presence of a nonionic surfactant such as the tween 40. Thus, the hydrophobic  $\beta$ -carotene is, in fact, right inside the hydrophobic core of mixed micelles made of tween and linoleic acid and reacts with free radicals arising from the autoxidation. The decrease of  $\beta$ -carotene absorbance resulting from this oxidation is followed by UV/VIS spectroscopy ( $\lambda = 464$  nm). Although numerous works have been published on the kinetics of radical scavenging [13–18], it is still necessary to find a parameter that allows the antioxidant evaluation for the  $\beta$ -carotene bleaching method.

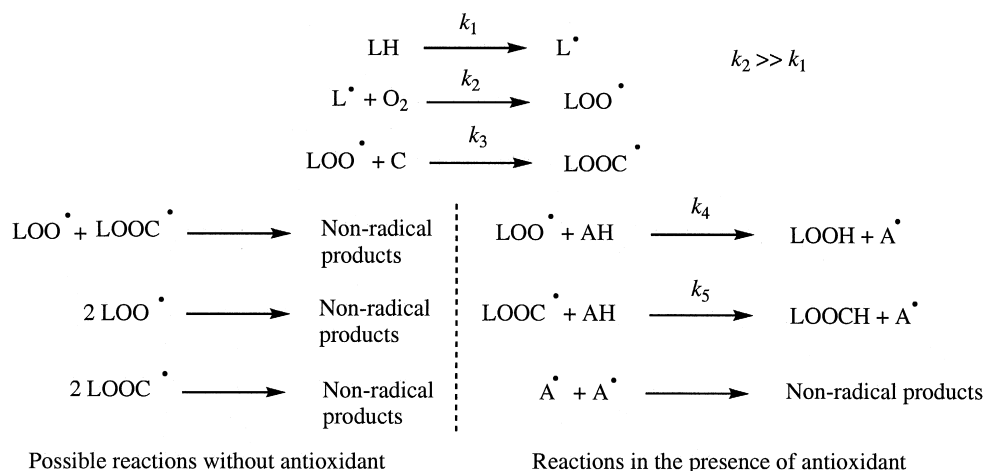
It is noteworthy that there are several ways to quantify the antioxidant activities. For instance, *Cuvelier et al.* [19] calculated parameters such as Antioxidant Activity Index (AAI) and/or Antioxidant Activity Coefficient (AAC). The former is based on the half reaction time, and the latter gives the activity of a compound at the end of the reaction.

However, since these calculations are not the only way to determine activities, it seems quite difficult to compare results from different works. So, we will first propose a kinetic parameter to quantify the antioxidant activity. To this end, keeping in mind the conditions used in this test (*cf. Exper. Part*), it seems interesting to specify the kinetics of  $\beta$ -carotene oxidation.

Without antioxidant, the lipid radical formation is not limited, and the velocity of the reaction is high. In the presence of an antioxidant, the free-radical formation is inhibited, and the bleaching phenomenon occurs more slowly. The consumption rate of  $\beta$ -carotene could be correlated to the antioxidant activity of our molecules.

In the absence of an antioxidant, the oxidation reactions occurring in solution can be described as shown in *Scheme 1* [2].

Scheme 1. Possible Reactions with and without Antioxidant ( $k_1, k_2, k_3, k_4$ , and  $k_5$  are reaction rates, LH = linoleic acid, C =  $\beta$ -carotene)



In this mechanism,  $\beta$ -carotene reacts with  $\text{LOO}^\bullet$  species [7]. At the beginning of the reaction, one can consider that all free radicals  $\text{LOO}^\bullet$  lead to  $\text{LOOC}^\bullet$ . This would be true as long as  $[\text{C}]$  is much greater than  $[\text{LOO}^\bullet]$ . Under these conditions, the rate  $v$  of  $\beta$ -carotene consumption is given by the following equation:

$$v = -d[\text{C}]/dt = k_3[\text{C}][\text{LOO}^\bullet]$$

Applying the stationary-state conditions and by assuming the concentration of oxygen much higher than  $[\text{L}^\bullet]$  and the rate of peroxidation greater than the formation of  $\text{L}^\bullet$ , we can write:

$$d[\text{L}^\bullet]/dt = 0 = k_1[\text{LH}] - k_2[\text{L}^\bullet][\text{O}_2] \quad (1)$$

$$d[\text{LOO}^\bullet]/dt = 0 = k_2[\text{L}^\bullet][\text{O}_2] - k_3[\text{LOO}^\bullet][\text{C}] \quad (2)$$

According to *Eqns. 1* and *2*, with  $k_1[\text{LH}] = v_1$ :

$$k_3[\text{LOO}^\bullet][\text{C}] = k_2[\text{L}^\bullet][\text{O}_2] = k_1[\text{LH}] = v_1$$

Therefore

$$v = -d[\text{C}]/dt = k_1[\text{LH}]$$

Close to the beginning of the reaction, one can consider that  $[\text{LH}] \approx [\text{LH}]_0 \approx \text{constant}$ , and  $[\text{LH}] \gg [\text{L}^\bullet]$ . Thus, by integrating the above expression between 0 and  $t$ , one obtains:

$$[C]_0 - [C] = k_1[LH]_0 \cdot t \text{ or } ([C]_0 - [C])/[C]_0 = (k_1[LH]_0/[C]_0) \cdot t$$

$$\text{or } 1 - [C]/[C]_0 = K_0 \cdot t \text{ with } k_1[LH]_0/[C]_0 = K_0$$

Because the absorbance  $A$  in UV/VIS is proportional to  $[C]$ :

$$A/A_0 = 1 - K_0 \cdot t$$

where  $K_0$  represents the slope of  $A/A_0$  vs. time of the reaction in the absence of antioxidant. At the beginning of the reaction, a plot of  $A/A_0$  vs. time must give a linear decrease, which is actually observed (Fig. 1).

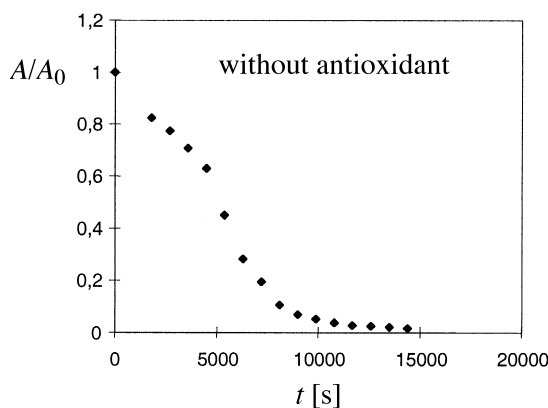


Fig. 1. Plot of  $A/A_0$  vs. time

In the presence of an efficient antioxidant and according to the equations shown in *Scheme 1*, the rate of  $\beta$ -carotene consumption, during the early stages of the oxidation, is given by

$$v = -d[C]/dt = k_3[C][LOO^\bullet].$$

Keeping in mind the previous remarks and assuming that the concentration of antioxidant  $[AH]$  is high at the beginning of the reaction, the following expressions can be obtained for the velocity of the reaction:

$$d[LOO^\bullet]/dt = 0 = v_1 - k_3[LOO^\bullet][C] - k_4[LOO^\bullet][AH]$$

from which one may conclude that  $[LOO^\bullet] = k_1[LH]_0/(k_3[C] + k_4[AH])$

$$v = -d[C]/dt = (k_3[C] k_1[LH]_0)/(k_3[C] + k_4[AH]) = k_1[LH]_0/(1 + k_4[AH]/k_3[C])$$

At this point, two hypotheses can be considered, if  $[AH]$  and  $[C]$  are in the same range of concentration:

1) If  $k_3$  is greater than  $k_4$ , the antioxidant is a spin trap less efficient than  $\beta$ -carotene and  $\text{LOO}\cdot$  reacts with  $\beta$ -carotene. Thus,  $1 \gg k_4[\text{AH}]/k_3[\text{C}]$  and  $v = v_1 = k_1[\text{LH}]_0$ , therefore

$$[\text{C}]/[\text{C}]_0 = 1 - K_0 \cdot t$$

2) If  $k_3$  is much smaller than  $k_4$ , the antioxidant is more efficient than  $\beta$ -carotene and 1 is negligible compared to  $k_4[\text{AH}]/k_3[\text{C}]$ . The velocity of the reaction becomes:

$$v = -d[\text{C}]/dt = k_1[\text{LH}]_0 k_3[\text{C}]/k_4[\text{AH}]$$

We can write:

$$v_1 k_3 = v_1 k_1[\text{LH}]_0 k_3 = K'$$

At the beginning of the reaction, one can assume that  $[\text{AH}] \approx [\text{AH}]_0$ , thus:

$$v = -d[\text{C}]/dt = K'[\text{C}]/k_4[\text{AH}]_0$$

$$\ln [\text{C}_0]/[\text{C}] = (K'/k_4[\text{AH}]_0) \cdot t$$

Consequently,

$$\ln (A_0/A) = (K'/k_4[\text{AH}]_0) \cdot t = K'' \cdot t \text{ with } K'' = (k_1 k_3 [\text{LH}]_0)/(k_4[\text{AH}]_0)$$

where  $K''$  represents the slope of  $\ln (A_0/A)$  vs. reaction time in the presence of antioxidant.

By measuring the slopes of  $\ln (A_0/A)$  vs.  $t$  at the initial conditions, we can obtain a significant value of the antioxidant activity of AH. In this case, the antioxidant efficiency decreases when the slope ( $K''$ ) increases.

The ratio between the slopes obtained in the absence or in the presence of antioxidant,  $K_0$  and  $K''$ , respectively, is

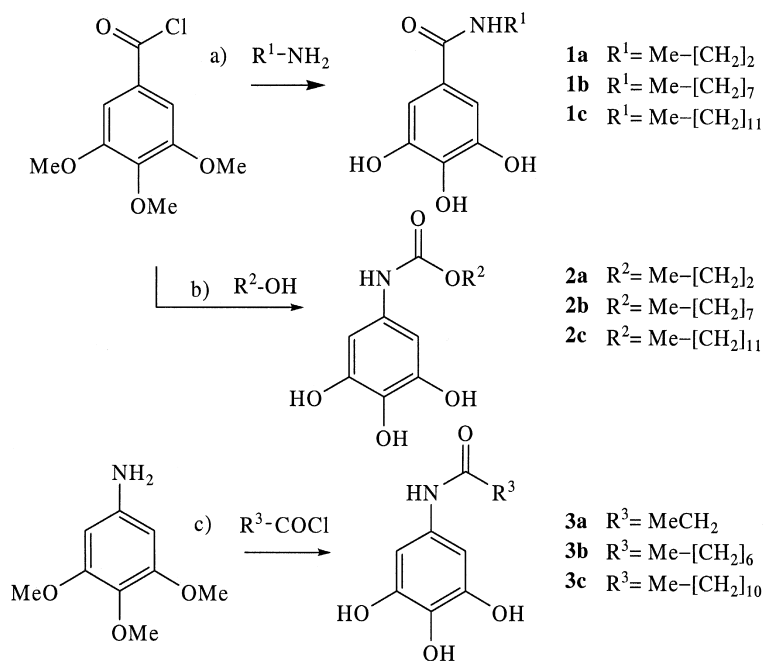
$$K = (k_1[\text{LH}]_0/[\text{C}_0]) \cdot (k_4[\text{AH}]_0/[k_1 \cdot k_3 [\text{LH}]_0]) = K_0/K''$$

So, the efficiency of an antioxidant under the chosen conditions will be specified by its  $K$  value. The higher the  $K$  value is, the better is the antioxidant activity.

**Results and Discussion.** – *Synthesis.* To study the impact of the chemical structure on the antioxidant activity in organized media, a series of models derived from gallic acid were designed. The synthesis and the compounds prepared are depicted.

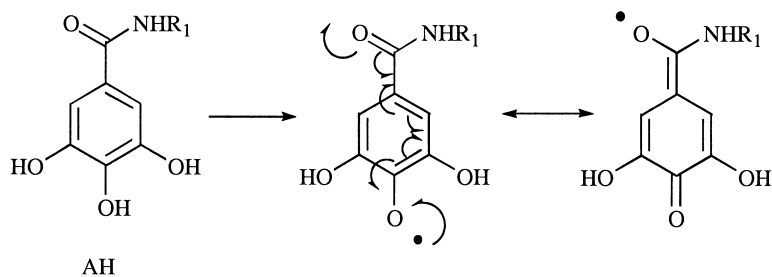
1) The first family is composed of hydrophobic compounds. In this case, two structural parameters were chosen: *i*) the nature of the link between the polyphenol and the hydrocarbon chain, *ii*) the tail length of the hydrocarbon.

The stability of the phenoxy radical formed during the oxidation process probably depends on the electron delocalization. Consequently, the function involved in the

Scheme 2. *Synthesis of Hydrophobic Gallic Acid Derivatives*

a) 1.  $\text{Et}_3\text{N}/\text{CH}_2\text{Cl}_2$ ; 72–91%; 2.  $\text{Br}_3\text{B}/\text{CH}_2\text{Cl}_2$ ; 20–96%. b) 1.  $\text{NaN}_3$ , acetone/ $\text{H}_2\text{O}$ ,  $\Delta$ , DABCO/toluene; 82–91%; 2.  $\text{Br}_3\text{B}/\text{CH}_2\text{Cl}_2$ , *IRC50* resin; 20–96%. c) 1.  $\text{Et}_3\text{N}/\text{CH}_2\text{Cl}_2$ ; 87–92%; 2.  $\text{Br}_3\text{B}/\text{CH}_2\text{Cl}_2$ ; 20–96%.

connecting part may have an impact on the free-radical stability and the antioxidant activity. For instance, an electron-withdrawing group will decrease the electron density in the aromatic ring and tend to stabilize the free radical formed (*Scheme 3*). On the contrary, electron-releasing groups will tend to decrease the phenoxy stability. Such mesomeric effects may have a non-negligible effect on the intrinsic antioxidant properties.

Scheme 3. *Stabilization of the Phenoxy Radical by Electron Delocalization*

To evaluate the relation between the hydrophobic character and the antioxidant activity, different chain lengths were simply grafted to the polyphenolic unit. Thus, three different functions, *i.e.*, *N*-alkyl amide (compounds **1a–1c**) [20][21], carbamate (compounds **2a–2c**), and *N*-phenyl amide (compounds **3a–3c**) are used to graft the polyphenol moiety on the hydrophobic chains (*Scheme 2*). Products **1a–1c** were prepared from trimethoxygallic acid activated as acid chloride. Reactions were performed in  $\text{CH}_2\text{Cl}_2$  in the presence of  $\text{Et}_3\text{N}$ . Carbamates **2a–2c** were synthesized starting from the same material and  $\text{NaN}_3$  in acetone/ $\text{H}_2\text{O}$  50:50. In this case, the reactions between isocyanate intermediate and alcohols were performed in toluene under basic conditions in the presence of a catalytic amount of 1,4-diazabicyclo[2.2.2]octane (DABCO). *N*-Phenyl amide derivatives **3a–3c** were obtained from 3,4,5-trimethoxyaniline. Hydrolysis of the MeO groups was finally achieved by using  $\text{BBr}_3$  in  $\text{CH}_2\text{Cl}_2$ .

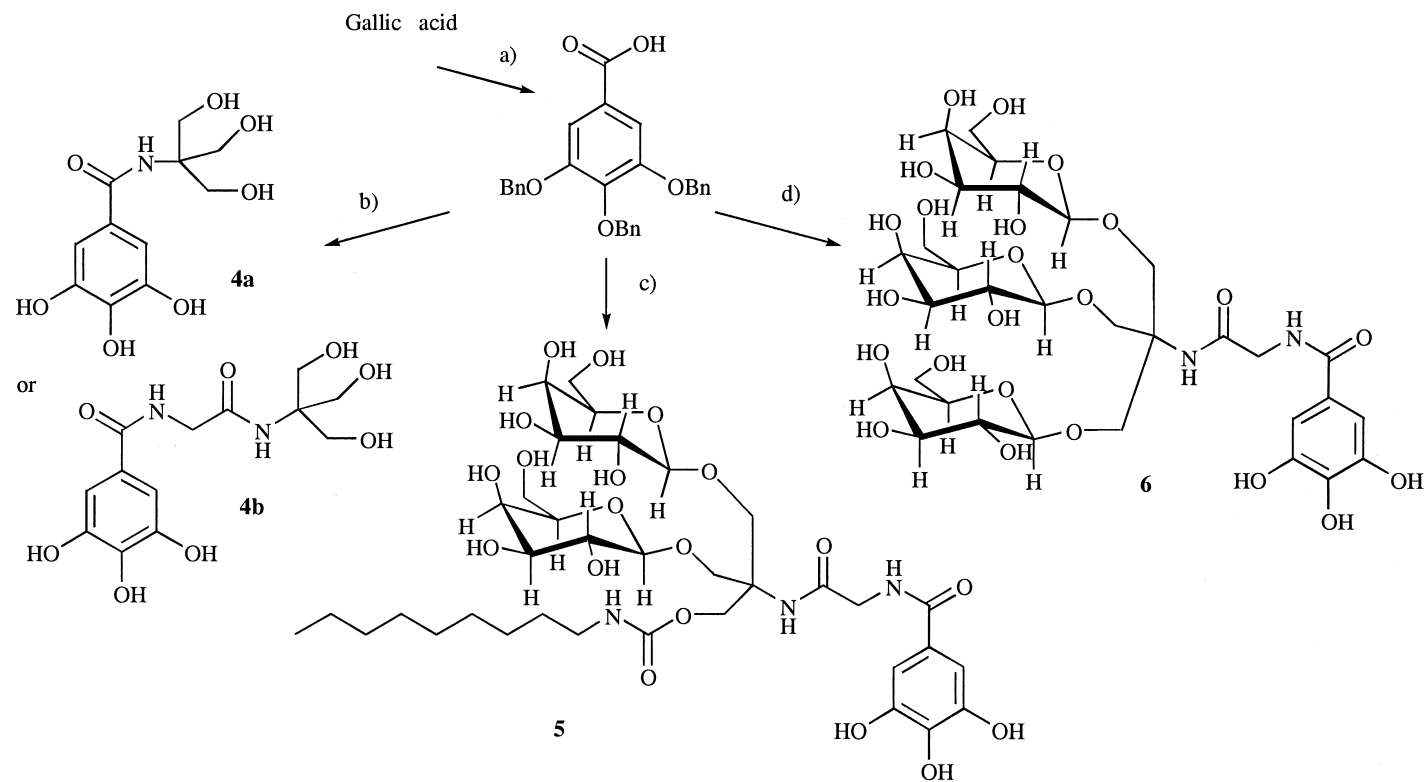
2) The lipophilic character of gallic acid was modified in a second approach. This parameter is assumed to have an impact on the polyphenol localization in an organized system. Indeed, a hydrophilic moiety will tend to drive the molecules preferentially into the aqueous phase, whereas a hydrophobic tail will maintain the molecules in the micellar aggregates containing the lipid substrates. For example, one can expect that an amphiphilic structure with a polyphenol group may form mixed micelles with lipids and, thus, inhibit the autoxidation phenomenon.

Grafting a tris(hydroxymethyl)aminomethane (*Tris*)-derived residue onto the gallic acid architecture provides either hydrophilic compounds (products **4a**, **4b**, and **6**) or amphiphilic compound **5** (*Scheme 4*). The latter, **5**, bears on a *Tris* moiety two  $\beta$ -D-galactopyranose groups, which provide the hydrophilic character to the molecule, whereas a hydrocarbon chain with nine C-atoms was chosen to provide the lipophilic part. In a previous work, such a model, used to modify the bioavailability of spin-trap compounds, showed its efficiency in membrane crossing [22]. Coupling reactions between gallic acid and *Tris* derivatives [22][23] were performed in the presence of either 2-ethoxy-*N*-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ) or (benzotriazol-1-yl)oxytris(dimethylamino)phosphonium hexafluorophosphate/4-(dimethylamino)-pyridine (BOP/DMAP) to lead to the products **4a**, **4b**, **5**, and **6** after hydrogenolysis ( $\text{H}_2$ , Pd/C).

3) To evaluate the relation between the number of polyphenol moieties per molecule and the antioxidant activity, a definite number of polyphenol can be attached to the same molecule. Thus, two or three pentafluorophenyl gallic acid esters (previously synthesized by using pentafluorophenol and 1,3-dicyclohexylcarbodiimide (DCC) as coupling reagent) were grafted to *i*) 1,3-diaminopropan-2-ol, *ii*) tris(aminoethyl)amine, and *iii*) *Tris*-derived cotelomer [24] to give polygallate structures **7**, **8**, and **9**, respectively (*Scheme 5*).

*Antioxidant Efficiency.* To classify antioxidants according to their effectiveness, we determined for each compound its *K* value. Indeed, since all antioxidants are tested under the same conditions, *i.e.*, with a constant concentration  $[\text{AH}]_0$ , we can consider that  $[\text{AH}]_0/k_3$  is constant for all experiments. On the other hand,  $\beta$ -carotene can act as an antioxidant by trapping lipid radicals in chain-termination reactions [25]. This observation enables us to choose the  $\beta$ -carotene as an antioxidative reference. So, we can estimate the efficiency of a given antioxidant compared to  $\beta$ -carotene; the

Scheme 4. Synthesis of Hydrophilic Gallic Acid Derivatives



a) 1. PhCH<sub>2</sub>Br, acetone; 2. KOH/MeOH; 45%. b) 1. EEDQ in MeOH, coupling of *Tris* leads to **4a**, coupling of *N*-[tris(hydroxymethyl)methyl]glycinamide [23] (Gly-*Tris*) gives **4b**; 17–40%; 2. H<sub>2</sub>, Pd/C; 70%. c) 1. Bis[*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactosyl)hydroxymethyl]nonylamidocarbonyloxymethyl [22], BOP/DMAPI in CH<sub>2</sub>Cl<sub>2</sub>; 2. MeOH/MeONa; 3. H<sub>2</sub>, Pd/C; overall yield 59%. d) 1. *N*-[tris[*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactosyl)hydroxymethyl]methyl]glycinamide [23], BOP/DMAPI in CH<sub>2</sub>Cl<sub>2</sub>; 53%; 2. MeOH/MeONa; 98%; 3. H<sub>2</sub>, Pd/C; 54%.

The reaction scheme illustrates the synthesis of three compounds from gallic acid:

- Gallic acid** (3,4,5-trihydroxybenzoic acid) is the starting material.
- Reaction (a):** Gallic acid reacts with 2,3,4,5-tetrafluorobenzoic acid to form an intermediate ester.
- Reaction (b):** The intermediate ester reacts with 1,2-ethanediol to form compound **7**, which is a bis-ester of gallic acid and 1,2-ethanediol.
- Reaction (c):** The intermediate ester reacts with 1,2-ethanediol to form compound **8**, which is a bis-ester of gallic acid and 1,2-ethanediol.
- Reaction (d):** The intermediate ester reacts with a long-chain alkyl thiol,  $C_{12}H_{25}-S-(CH_2-CH)_n-H$ , to form compound **9**, which is a mono-ester of gallic acid and the long-chain alkyl thiol.

a) Pentafluorophenol/DCC in  $\text{CH}_2\text{Cl}_2$ ; 92%. b) 1.  $\text{Et}_3\text{N}/\text{CH}_2\text{Cl}_2$ ; 1,3-diaminopropan-2-ol; 30%; 2.  $\text{H}_2$ , Pd/C; 55%. c) 1. Pyridine, DABCO, tris(aminoethyl)amine; 50%; 2.  $\text{H}_2$ , Pd/C; 65%. d) 1. Pyridine, DABCO, cotelomer [24]; 2. MeOH/MeONa; 3.  $\text{H}_2$ , Pd/C; yield (weight) 92%.

antioxidant effectiveness is, therefore, directly proportional to  $K$ . High values of  $K$  imply strong antioxidant activities (compared to  $\beta$ -carotene). When  $K$  is smaller than 1,  $\beta$ -carotene is more powerful than the molecule tested.

It must be underlined that this test exhibits several advantages. First, kinetics of oxidation was recorded by plotting the sample absorbance vs. time. An example of these experiments with hydrophobic  $N$ -alkyl amide derivatives is shown in Fig. 2. A plot of  $\ln(A_0/A)$  vs. time allows calculation of the  $K$  values and the antioxidant efficiency by using the slopes at the beginning of the reactions (Fig. 3). The data obtained are collected in the Table.

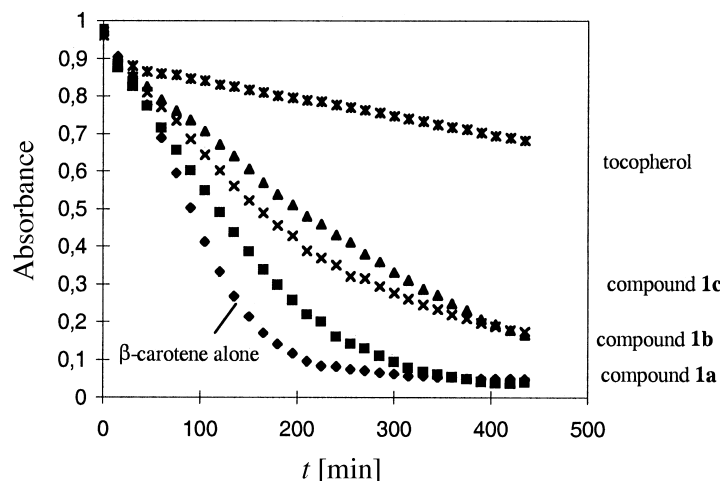


Fig. 2. Kinetics of the  $\beta$ -carotene oxidation in the presence of  $N$ -alkyl amides **1a**, **1b**, and **1c**, or  $\alpha$ -tocopherol

The test is simple. It is also easy to achieve, and one can measure the antioxidant activity of a compound within a few hours (at the beginning of the reaction).

A parameter such as  $K$  seems to be suitable for this type of kinetics, because it allows correlation of the reaction rates and concentrations. In addition, it can be used to compare results from different experiments. 1) Although we cannot work at room temperature (the reaction rates are too low), the conditions of oxidation are quite mild ( $50^\circ$ ). The products involved should not undergo any alteration. 2) Linoleic acid is very often found in foodstuffs, and final solutions are prepared in EtOH, and our derivatives are all soluble in this solvent. However, one can note that, although linoleic acid is a widespread fatty acid, its behavior cannot be extended to the whole family of lipids, which is rather complex. For instance, hydrophobic fatty acid esters usually contain glycerol derivatives, and their aqueous behavior cannot be compared with free fatty acids, which may form micelles because of their amphiphilic character.

The antioxidant activity of the 16 gallic acid derivatives prepared in this work were compared to those observed with known antioxidants such as  $\alpha$ -tocopherol, trolox, BHA, BHT and commercial alkyl gallates. Based on the different  $K$  values obtained, these results can be interpreted as follows: 1) First, the starting polyphenolic structure, *i.e.*, gallic acid, is not active in this medium. In contrast,  $\alpha$ -tocopherol, Trolox, BHT, and

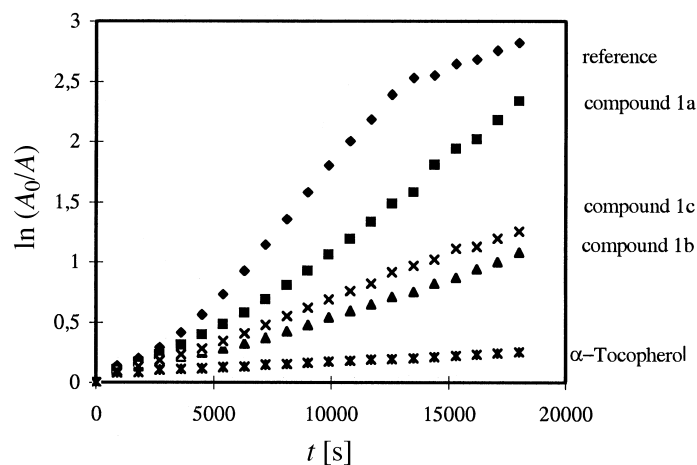


Fig. 3. Determination of  $K = K_0/K$  by using the slopes at the beginning of reactions for **1a**, **1b**, and **1c**, and  $\alpha$ -tocopherol ( $K$ ) and without antioxidant ( $K_0$ )

Table. Values of  $K$  Obtained for Antioxidants at a Concentration of  $3.52 \mu\text{M}$

| Compounds            | $K$ (corrected) | Activity <sup>a)</sup> |
|----------------------|-----------------|------------------------|
| Gallic acid          | 1               | —                      |
| Trolox <sup>®</sup>  | 45              | ++++                   |
| $\alpha$ -Tocopherol | 48.8            | ++++                   |
| BHA                  | 34              | +++                    |
| BHT                  | 38.6            | +++                    |
| Propyl gallate       | 4               | +                      |
| Octyl gallate        | 6.5             | +                      |
| Lauryl gallate       | 6               | +                      |
| <b>1a</b>            | 4               | +                      |
| <b>1b</b>            | 6.5             | +                      |
| <b>1c</b>            | 6               | +                      |
| <b>3a</b>            | 1               | —                      |
| <b>3b</b>            | 1               | —                      |
| <b>3c</b>            | 2               | —                      |
| <b>2a</b>            | 1               | —                      |
| <b>2b</b>            | 1               | —                      |
| <b>2c</b>            | 1               | —                      |
| <b>4a</b>            | 10              | ++                     |
| <b>4b</b>            | 15              | ++                     |
| <b>6</b>             | 2.5             | +                      |
| <b>5</b>             | 34              | +++                    |
| <b>7</b>             | 34              | +++                    |
| <b>8</b>             | 36              | +++                    |
| <b>9</b>             | —               | —                      |

<sup>a)</sup> To easily visualize the antioxidant efficiency, the following symbols were chosen; + indicates activity, whereas — means that the product is not active.

BHA, which were chosen as references, show strong activities. 2) Although they are less efficient than  $\alpha$ -tocopherol, alkyl gallates are antioxidants in this test. Similarly, *N*-alkyl amides **1a**, **1b**, and **1c** exhibit an antioxidant activity. 3) However, hydrophobic carbamates **2a**, **2b**, and **2c**, and *N*-phenyl amides (**3a**, **3b**, **3c**) are not efficient in this medium, such a result seems to show that the linkage plays a determining role in the stability of the phenoxy radical ( $A^\bullet$ ) formed during the chain-breaking reactions. This lack of activity could be attributed intrinsically to their structure. The linkage is clearly of great importance. Among possible explanations, the loss of stabilization of the radical formed during the reaction seems to be conceivable. One can assume that stabilizing effects increase the antioxidant activity of a given gallic acid derivative. Indeed, an electron-withdrawing function such as the C=O group of *N*-alkyl amides would decrease the electron density on the aromatic ring and increase the free radical stability (compounds **1**). In the case of both carbamates (compounds **2**) and *N*-phenyl amides (compounds **3**), this electron stabilization is not possible since a N-atom separates the electron-withdrawing C=O group from the aromatic ring. These products show actually very weak antioxidant activities.

Another way to explain these non-activities was provided by the theoretical method recently reported by *Wright et al.* [26]. This procedure, based on density functional theory, is used for the calculation of the gas-phase bond dissociation enthalpy (BDE) of the O–H bond and ionization potential (IP) for phenolic antioxidants. Although this method would require introduction of a solvent model into the calculations, simulations with this preliminary theoretical method could be used to predict the activities of candidate molecules. However, once a free radical is formed, another point must be taken into account: the stabilization phenomenon that depends on the well-known push-pull effect. Thus, the free-radical formation should be seen as the net result of several parameters, *i.e.*, BDE, IP, and push-pull effect.

Likewise, even if di- and trigalloyl compounds **7** and **8**, respectively, are quite efficient in emulsion, they do not show any synergistic effect compared to their hydrophobic mono-amide or ester analogues. Moreover, cotelomer **9** and other telomers (data not shown) bearing many galloyl groups are surprisingly poorly active. To explain these results, it seems necessary to take into account the physico-chemical properties of each molecule, and the nature of the medium in which the reaction is performed.

Indeed, one can note that both linoleic acid and  $\beta$ -carotene are lipophilic, and consequently, they will partition preferentially within micelles. According to *Porter et al.* [4], in an aqueous medium, hydrophilic molecules will be less active than hydrophobic or amphiphilic derivatives. This hydrophobic character will drive them into the core of micelles where they will act as antioxidants. Therefore, to be efficient against the oxidation in this emulsified medium, our molecules should possess either a lipophilic or an amphiphilic character.

Gallic acid is soluble in  $H_2O$  (11 mg/ml), and it will be located mainly in the  $H_2O$  phase. Thus, because it is not close to linoleic acid, it can not protect this compound against the oxidation phenomenon. *Porter's* hypothesis is also verified in the case of esters and our *N*-alkyl amide derivatives, which all bear hydrophobic tails. The best efficiency is observed, when the chains are long enough to provide a sufficient lipophilic character. For instance, an increase in activity is observed when the chain length is

enhanced from C<sub>3</sub> (compound **1a**,  $K = 4$ , Table) to C<sub>8</sub> (compound **1b**,  $K = 6.5$ , Table). The amphiphilic criterion can also be illustrated with derivative **5**. In addition to the hydrophilic galactose units, this molecule bears a C<sub>9</sub> tail. Thus, one can assume that this amphiphile would form mixed micelles with nonionic tween and consequently would carry the polyphenol moiety in contact with the oxidizable substrates. Effectively, its activity is the best one observed in the family of *Tris* derivatives (compound **5**,  $K = 34$ , Table).

In the same way, keeping in mind that phenolic moieties are rather hydrophobic groups (because of intramolecular H-bonds), both di- and trigalloyl compounds, **7** and **8**, respectively, can be considered as hydrophobic. Therefore, they would be located within the micelle core and they could avoid lipid oxidation. Indeed, they exhibit good activities.

On the contrary, when gallic acid is grafted onto hydrophilic architectures such as *Tris*, the resulting products can be seen as weak amphiphilic molecules with a strong hydrophilicity, the gallic acid moiety representing the hydrophobic part of the molecule. Thus, the weak activities observed for **4a**, **4b**, and **6** could be explained by the whole hydrophilic character of the molecule.

The same explanation could be provided for the telomer **9**, which seems to exhibit no antioxidant activity. The telomer structure displays an amphiphilic character, and this type of compound was previously used to extract membrane proteins or to vectorize drugs [27–29]. However, in the present case, the hydrophilic part of the telomer is large and bears gallic units; consequently, the polyphenols would be brought outside the micelles in the H<sub>2</sub>O phase far away from the oxidizable lipidic substrates.

Finally, concerning the standards used in this tests, all derivatives are hydrophobic or amphiphilic, and they are located in the lipid phase. BHA, BHT, and  $\alpha$ -tocopherol are hydrophobic, so they are in contact with the lipid substrates and avoid the oxidation reactions. *Trolox* exhibits an amphiphilic character and inserts into micelles. It was actually previously observed that this antioxidant goes mainly into micelles made of tween, and a very weak amount is in the aqueous phase [20].

**Conclusions.** – We have seen that the antioxidant activity of a given compound depends on its structure and, more precisely, on its ability to stabilize the radical formed. So, the nature of the bond between the polyphenol group and the rest of the molecule is quite important. In all cases, a push-pull effect is needed. Moreover, since the antioxidant power is also closely connected to the oxidation medium, the hydrophilic or lipophilic character of the active principle drastically affects its antioxidant activity. The results obtained are in accordance with the polar paradox; hydrophobic derivatives inhibit oxidation of lipidic compounds in an aqueous phase, whereas hydrophilic products are not efficient. One can underline that oxidation is mainly an interfacial phenomenon. Thus, the optimal efficiency of an antioxidant will be obtained when this molecule shows a peculiar affinity to the air-oil or air-solvent interfaces in lipidic media, *i.e.*, when it possesses a well-adapted amphiphilic character. Similarly, in the case of a micellar system, the affinity to the O/W interface is necessary to obtain an antioxidant effect. These interfacial phenomena are key points and must be taken into account and further studied to better understand the antioxidant activities in heterogeneous or lipidic media such as foodstuffs.

To increase the antioxidant efficiency of the whole molecule and to eventually introduce a clustering effect, we are currently developing the synthesis of a new kind of amphiphilic dendrimer or telomer structures, which bear several gallic acid units in their hydrophobic part. Beside this work, we are also studying this antioxidant efficiency through oxidative systems, which containing either synthetic membrane bilayers or liposomes, by considering the physiological location of the oxidative process and the key role of the membrane crossing of a given compound.

### Experimental Part

**General.** Unless noted otherwise, all starting materials were obtained from commercial suppliers and were used without further purification; the solvents were redistilled on  $\text{CaCl}_2$ ,  $\text{CaH}_2$ , KOH, or Na according to the solvent used. M.p.: an electrothermal 9100 apparatus; uncorrected. Anal. TLC: Merck silica-gel 60  $F_{254}$  plates. All compounds were characterized by their anal. and spectroscopic data such as UV/VIS (Varian), IR (FTIR Mattson 1000),  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR (Bruker AC 250,  $^1\text{H}$ : at 250 MHz and  $^{13}\text{C}$ : at 62.86 MHz), and mass spectrometry (Jeol DX 100 in FAB $^+$ ). In NMR, chemical shifts ( $\delta$ ) are given in ppm relative to TMS with the D signal of the solvent ( $\text{CDCl}_3$  or  $(\text{D}_6)\text{DMSO}$ ) as a heteronuclear reference for  $^1\text{H}$  and  $^{13}\text{C}$ . In  $^1\text{H}$ -NMR, coupling constants  $J$  are given in Hz. Microanalyses were carried out at CNRS Verneson (France). The antioxidant-activity measurements were recorded on a colorimeter Milton Roy spectrometric 20D.

**Syntheses.** N-Alkyl-3,4,5-trihydroxybenzamides **1a–1c** [21] [30]. A typical procedure is described for 3,4,5-trihydroxy-N-propylbenzamide **1a**. The preparation of **1b** and **1c** follows the same procedure. All derivatives were fully characterized.

$\text{PrNH}_2$  (1.18 ml, 0.85 g, 14.30 mmol, 1.1 equiv.) was solubilized in 20 ml of freshly distilled  $\text{CH}_2\text{Cl}_2$ .  $\text{Et}_3\text{N}$  (2 ml, 1.45 g, 14.30 mmol, 1.1 equiv.) was added to this soln. 3,4,5-Trimethoxybenzoyl chloride (3.00 g, 13.00 mmol, 1 equiv.) in 10 ml of distilled  $\text{CH}_2\text{Cl}_2$  was poured dropwise under  $\text{N}_2$  into the soln. with cooling ( $5-10^\circ$ ). The mixture was stirred for 12 h at r.t. The solvent was removed under reduced pressure. The resulting crude material was then dissolved in 40 ml of AcOEt. The org. phase was washed successively 3  $\times$  with 1N HCl, 10 ml of 10%  $\text{Na}_2\text{CO}_3$ , and 10 ml of brine. The org. layer was dried ( $\text{Na}_2\text{SO}_4$ ). After purification on a silica-gel column (AcOEt/hexane 6:4), 3.06 g (12.10 mmol; 93%) of the trimethoxy derivative was isolated. M.p.  $118-118.5^\circ$ . UV/VIS (MeOH):  $\lambda_{\text{max}}$  272.7 (5960). IR (KBr): 3301 (N–H), 1631 (C=O), 713 (N–H), 1130 (C=O).  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ ): 12.07 (Me); 23.58 ( $\text{CH}_2$ ); 42.52 ( $\text{CH}_2$ ); 56.91 (*m*-MeO); 61.50 (*p*-MeO); 105.01, 131.02, 141.42, 153.79 (arom. C); 167.92 (CO). Anal. calc.: C 61.64, H 7.53, N 5.67%; found: C 61.89, H 7.53, N 5.67.

The trimethoxy derivative (1.73 g, 6.84 mmol, 1 equiv.) was solubilized in freshly distilled  $\text{CH}_2\text{Cl}_2$  (10 ml). Under cooling ( $5-10^\circ$ ), 1M  $\text{BBr}_3$  (41 ml, 41.00 mmol, 6 equiv.) was added slowly to the soln. The mixture was stirred for 2 h at r.t. After adding  $\text{H}_2\text{O}$  (20 ml), the mixture was stirred for few min, then the aq. layer was extracted with  $\text{Et}_2\text{O}$  ( $3 \times 10$  ml). The org. phases were collected, washed with brine ( $3 \times 10$  ml), dried ( $\text{Na}_2\text{SO}_4$ ), and concentrated under reduced pressure. The product was then dissolved in MeOH (20 ml) and acidified with an IRC50 $^\circ$  resin. After 1 h, the resin was filtered off, and MeOH was removed. After recrystallization (AcOEt/hexane), **1a** was obtained (187 mg, 13%). M.p.  $100^\circ$  (dec). UV/VIS (MeOH):  $\lambda_{\text{max}}$  273.8 (5390). IR (KBr): 3222 (O–H), 1585 (C=O), 725 (N–H).  $^1\text{H}$ -NMR ( $(\text{D}_6)\text{DMSO}$ ): 0.87 (*t*,  $J = 7.3$ , Me); 1.50 (*s*,  $J = 7.3$ ,  $\text{CH}_2$ ); 3.15 (*m*,  $\text{CH}_2$ ); 6.82 (*s*, 2 arom. H); 8.06 (*m*, NH).  $^{13}\text{C}$ -NMR ( $(\text{D}_6)\text{DMSO}$ ): 11.46 (Me); 22.52 ( $\text{CH}_2$ ); 27.97 ( $\text{CH}_2$ ); 106.62, 125.16, 135.95, 145.31; 166.25 (CO).

3,4,5-Trimethoxyphenyl Isocyanate.  $\text{NaN}_3$  (4.2 g, 65 mmol, 5 equiv.) was dissolved in  $\text{H}_2\text{O}$  (15 ml). 3,4,5-Trimethoxybenzoyl chloride (3 g, 13.00 mmol, 1 equiv.) in acetone (25 ml) was added dropwise under cooling ( $8-10^\circ$ ) to the soln., which was stirred for 1 h. The mixture was extracted quickly 3  $\times$  with cyclohexane (25 ml), washed with cold  $\text{H}_2\text{O}$  (20 ml), dried ( $\text{Na}_2\text{SO}_4$ ), and poured slowly under  $\text{N}_2$  into boiling cyclohexane. After distillation of cyclohexane, the expected isocyanate (2.58 g, 12.34 mmol, 95%) was isolated. IR (KBr): 2273 (N=C=O), 2148 (N=C=O; Fermi resonance).  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ ): 3.81 (*s*, *p*-MeO); 3.83 (*s*, 2 *m*-MeO); 6.32 (*s*, 2 arom. H).  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ ): 56.91 (*m*-MeO); 61.62 (*p*-MeO); 107.35, 126.15, 153.70 (arom. C); 172.42 (NCO).

Alkyl N-(3,4,5-Trihydroxyphenyl)carbamates **2a–2c**. A typical procedure is presented for propyl N-(3,4,5-trihydroxyphenyl)carbamate (**2a**). Preparation of **2b** and **2c** follows the same procedure. All carbamates were fully characterized.

3,4,5-Trimethoxyphenyl isocyanate (1 g, 4.80 mmol, 1.1 equiv.) and *PrOH* (0.33 ml, 264 mg, 4.40 mmol, 1 equiv.) were dissolved in anhyd. toluene (40 ml) and refluxed for 2 h. The solvent was then removed, and the resulting crude material was purified on silica gel (AcOEt/hexane 3:7) to yield the trimethoxy derivative (1.07 g, 4.00 mmol, 91%). M.p. 90–91°. UV/VIS (MeOH):  $\lambda_{\text{max}}$  273.0 (950). IR (KBr): 3342 (N–H), 1720 (C=O), 660 (N–H), 1128 (C–O). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 0.98 (t, *J* = 7.25, Me); 1.69 (s, *J* = 7.25, CH<sub>2</sub>); 3.81 (s, *p*-MeO); 3.84 (s, 2 *m*-MeO); 4.12 (t, *J* = 6.80, CH<sub>2</sub>O); 6.63 (m, NH); 6.69 (s, 2 H<sub>o</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>): 10.98 (Me); 22.94 (CH<sub>2</sub>); 56.73 (*m*-MeO); 61.63 (*p*-MeO); 67.52 (CH<sub>2</sub>); 97.02, 134.82, 154.11 (arom. C); 154.30 (CO). Anal. calc.: C 57.98, H 7.11, N 5.20, found: C 58.28, H 7.16, N 5.30.

Cleavage of MeO groups was performed as described for **1a**. The protected compound (1.88 g, 7.00 mmol, 1 equiv.) and 1M BBr<sub>3</sub> (42 ml, 42.00 mmol, 6 equiv.) lead, after recrystallization in AcOEt/hexane, to **2a** (1.45 g, 6.39 mmol, 91%). M.p. 55–55.2°. UV/VIS (MeOH):  $\lambda_{\text{max}}$  281.0 (3210). IR (KBr): 3300 (O–H), 1701 (C=O), 669 (N–H). <sup>1</sup>H-NMR ((D<sub>6</sub>)DMSO): 0.93 (t, *J* = 7.5, Me); 1.62 (m, *J* = 7.3, 6.5, CH<sub>2</sub>); 3.98 (t, *J* = 6.5, CH<sub>2</sub>); 6.47 (s, 2 H<sub>o</sub>); 9.10 (s, NH). <sup>13</sup>C-NMR ((D<sub>6</sub>)DMSO): 10.26 (Me); 21.94 (CH<sub>2</sub>); 65.21 (CH<sub>2</sub>O); 98.23, 128.33, 130.59, 145.97 (arom. C); 153.51 (CO).

N-(3',4',5'-Trihydroxyphenyl) Amides **3a–3c**. A typical procedure is presented for N-(3',4',5'-Trihydroxyphenyl)octanamide (**3b**).

3,4,5-Trimethoxyaniline (500 mg, 2.73 mmol, 1 equiv.) was dissolved in dist. CH<sub>2</sub>Cl<sub>2</sub> (30 ml). Et<sub>3</sub>N (0.4 ml, 276 mg, 2.73 mmol, 1 equiv.) was poured dropwise under N<sub>2</sub> and under cooling to a soln. of octanoyl chloride (0.52 ml, 488 mg, 3.00 mmol, 1.1 equiv.) in CH<sub>2</sub>Cl<sub>2</sub> (10 ml). After 12 h at r.t., the solvent was removed, and the crude oil was dissolved in AcOEt (40 ml). The org. phase was washed successively 3 × with 1N HCl (10 ml), aq. NaHCO<sub>3</sub> (10%, 10 ml), and dried (Na<sub>2</sub>SO<sub>4</sub>). The resulting material was purified on silica gel (AcOEt/hexane: 35:65) to yield, after recrystallization (MeOH/H<sub>2</sub>O), the expected product (480 mg, 1.55 mmol, 57%). M.p. 62–63°. UV/VIS (EtOH):  $\lambda_{\text{max}}$  273.0 (3730). IR (KBr): 3291 (N–H), 1655 (C=O), 723 (N–H), 1134 (C–O). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 0.89 (t, *J* = 7.5, Me); 1.26 (m, 4 CH<sub>2</sub>); 1.73 (m, CH<sub>2</sub>); 2.34 (t, *J* = 7.50, COCH<sub>2</sub>); 3.81 (s, *p*-MeO); 3.85 (s, 2 *m*-MeO); 6.84 (s, 2 H<sub>o</sub>); 7.05 (s, NH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>): 14.73 (Me); 23.30–32.37 (CH<sub>2</sub>); 38.58 (CH<sub>2</sub>); 56.85 (*m*-MeO); 61.65 (*p*-MeO); 98.19 (*o*-CH); 134.77, 154.07 (*m*-MeO); 172.50 (CO). Anal. calc.: C 65.99, H 8.80, N 4.53, found: C 66.27, H 9.3, N 4.09.

Cleavage of MeO groups was performed as described for **1a**. The trimethoxy derivative (1.65 g, 5.34 mmol, 1 equiv.) and 1M BBr<sub>3</sub> (32 ml, 32.00 mmol, 6 equiv.) lead to **3b** (1.00 g, 3.75 mmol, 70%). M.p. 135° (dec.). UV/VIS (EtOH):  $\lambda_{\text{max}}$  275.4 (3950). IR (KBr): 3330 (O–H), 1655 (C=O), 723 (N–H). <sup>1</sup>H-NMR ((D<sub>6</sub>)DMSO): 0.87 (t, *J* = 6.6, Me); 1.27 (m, 4 CH<sub>2</sub>); 1.56 (m, CH<sub>2</sub>); 2.22 (t, *J* = 7.3, CH<sub>2</sub>); 6.63 (s, 2 H<sub>o</sub>); 9.38 (s, NH). <sup>13</sup>C-NMR ((D<sub>6</sub>)DMSO): 13.94 (Me); 22.10–36.46 (6 CH<sub>2</sub>); 99.10 (*o*-CH); 128.87, 130.94, 145.80 (*m*-COH); 170.49 (CO).

3,4,5-Tribenzoyloxybenzoic Acid. Gallic acid (1 g, 5.88 mmol, 1 equiv.) was solubilized in dry acetone (60 ml). K<sub>2</sub>CO<sub>3</sub> (3.6 g, 25.88 mmol, 4.4 equiv.) and PhCH<sub>2</sub>Br (3.1 ml, 4.4 g, 25.88 mmol, 4.4 equiv.) were added to the soln. The mixture was heated under stirring for 12 h. After adding H<sub>2</sub>O (200 ml), the mixture was extracted 3 × with AcOEt. The solvent was removed *in vacuo*, and the residual oil was recrystallized (EtOH) to yield the perbenzylated derivative (2.48 g, 4.69 mmol, 80%). M.p. 93.5–94.5°. UV/VIS (MeOH):  $\lambda_{\text{max}}$  275.8 (10694). IR (KBr): 1711 (C=O), 1219 (C–O, ester), 1130 (C–O, ethers). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 5.12 (s, 3 CH<sub>2</sub>); 5.32 (s, COOCH<sub>2</sub>); 7.40 (m, 22 arom. H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>): 67.43 (COOCH<sub>2</sub>); 71.99 (*m*-CH<sub>2</sub>O); 75.82 (*p*-CH<sub>2</sub>O); 110.05 (*o*-CH); 125.84–153.23 (arom. C); 166.41 (CO).

The above benzyl ester (1 g, 1.89 mmol) was refluxed for 12 h in 3N KOH in MeOH (50 ml). The basic soln. was then acidified at r.t. with 3N HCl (pH 3–4). The resulting precipitate was filtered, dissolved in AcOEt (40 ml), washed 3 × with H<sub>2</sub>O (30 ml), and dried (Na<sub>2</sub>SO<sub>4</sub>). After recrystallization (MeOH), the product was isolated (357 mg, 0.81 mmol, 95%). M.p. 193.5–193.7°. UV/VIS (MeOH):  $\lambda_{\text{max}}$  272.6 (16038). IR (KBr): 1686 (C=O), 1431 (C–O, acid), 1128 (C=O, ethers). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 5.07 (s, *p*-CH<sub>2</sub>O); 5.08 (s, 2 *m*-CH<sub>2</sub>O); 7.30 (m, 17 arom. H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>): 71.99 (*m*-CH<sub>2</sub>O); 75.86 (*p*-CH<sub>2</sub>O); 110.43 (*o*-CH); 124.75 (CCOOH); 128.25–129.25 (arom. C); 137.26 (*m*-CCH<sub>2</sub>); 138.08 (*p*-CCH<sub>2</sub>); 143.94 (*p*-COCH<sub>2</sub>); 153.31 (*m*-COCH<sub>2</sub>); 171.15 (COOH).

3,4,5-Trihydroxy-N-[tris(hydroxymethyl)methyl]benzamide (**4a**). To a soln. of 3,4,5-tribenzoyloxybenzoic acid (1.00 g, 2.27 mmol, 1 equiv.) and EEDQ (620 mg, 2.50 mmol, 1.1 equiv.) in freshly distilled MeOH (40 ml), [tris(hydroxymethyl)amino]methane (300 mg, 2.50 mmol, 1.1 equiv.) was added. The mixture was heated under stirring for 12 h. Purification on silica gel (AcOEt) followed by recrystallization (AcOEt/hexane) gave the expected product (500 mg, 0.92 mmol, 95%). M.p. 129.2–129.5°. UV/VIS (MeOH):  $\lambda_{\text{max}}$  273.4 (17757). IR (KBr): 3325 (O–H), 1637 (C=O), 1122 (C–O, ether). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 3.71 (s, CH<sub>2</sub>OH); 4.61 (s, 3 OH (arom.)); 5.10 (s, *p*-CH<sub>2</sub>O); 5.13 (s, 2 *m*-CH<sub>2</sub>O); 7.20 (m, 17 H, *o*-CH, arom. H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>): 62.44 (quat. C); 63.24 (CH<sub>2</sub>OH); 72.17 (*m*-CH<sub>2</sub>O); 75.88 (*p*-CH<sub>2</sub>O); 107.75 (*p*-CH); 128.27–129.76 (CCONH, arom. C);

137.22 (CCH<sub>2</sub>O); 138.00 (CCH<sub>2</sub>O); 142.41 (*p*-COCH<sub>2</sub>); 153.51 (*m*-COCH<sub>2</sub>); 168.86 (CO). FAB<sup>+</sup>-MS: 544 ([*M* + H]<sup>+</sup>), 566 ([*M* + Na]<sup>+</sup>).

The tribenzyloxy derivative (400 mg, 0.74 mmol) was dissolved in MeOH. Pd/C (10%, 140 mg) was added to the mixture, and the resulting suspension was stirred under H<sub>2</sub> for 4 h. Pd was filtered off on *Millipore*, and MeOH was evaporated *in vacuo*. The resulting product was solubilized in H<sub>2</sub>O and then lyophilized to yield a white powder (134 mg, 0.50 mmol, 68%), which was stored under N<sub>2</sub>. M.p. 80° (dec.). UV/VIS (EtOH): λ<sub>max</sub> 275.8 (5965). IR (KBr): 3384 (O–H), 1697 (C=O), 1039 (C–O, phenols). <sup>1</sup>H-NMR (D<sub>2</sub>O): 3.65 (s, 3 OH); 3.79 (s, CH<sub>2</sub>OH); 6.81 (s, 2 H<sub>o</sub>). <sup>13</sup>C-NMR (D<sub>2</sub>O): 61.30 (quat. C); 62.85 (CH<sub>2</sub>OH); 108.30 (*o*-CH); 126.31 (CONH); 137.23 (*p*-COH); 145.40 (*m*-COH); 171.20 (CO).

N-(3',4',5'-Trihydroxybenzoyl)-N'-[tris(hydroxymethyl)methyl]glycinamide (**4b**). 3,4,5-Tri(benzyloxy)benzoic acid (1 g, 2.27 mmol, 1 equiv.) and EEDQ (620 mg, 2.50 mmol, 1.1 equiv.) were dissolved in freshly distilled EtOH (30 ml). N-[tris(hydroxymethyl)methyl]glycinamide [23] (445 mg, 2.50 mmol, 1.1 equiv.) was added to the soln. The mixture was then refluxed for 12 h. After purification on a silica-gel column (AcOEt/MeOH 9:1) and crystallization (MeOH/H<sub>2</sub>O), the expected product (235 mg, 0.39 mmol, 17%) was isolated. M.p. 185.2–186.2°. UV/VIS (EtOH): λ<sub>max</sub> 273.0 (6216). IR (KBr): 3319 (O–H), 1643 (C=O), 1126 (C–O, ethers). <sup>1</sup>H-NMR ((D<sub>6</sub>)DMSO): 3.58 (*d*, *J* = 5.50, 3 CH<sub>2</sub>OH); 3.94 (*d*, *J* = 5.80, CH<sub>2</sub>NH); 4.72 (*t*, *J* = 5.50, OH); 5.02 (s, *p*-CH<sub>2</sub>O); 5.19 (s, 2 *m*-CH<sub>2</sub>O); 7.38 (*m*, 18 H, *o*-CH, arom. H, NH (quat.)); 8.75 (*t*, *J* = 5.80, 1 H, NH(gly)). <sup>13</sup>C-NMR ((D<sub>6</sub>)DMSO): 43.05 (quat. C); 60.34 (CH<sub>2</sub>OH); 62.07 (CH<sub>2</sub>NH); 70.32 (CH<sub>2</sub>O); 106.56 (*o*-CH); 129.00 (CCO); 127.60–128.32 (arom. C); 136.80, 137.40 (CCH<sub>2</sub>O); 139.8 (*p*-C); 151.93 (*m*-C); 165.77 (CO); 169.75 (CONH). FAB<sup>+</sup>-MS: 601 ([*M* + H]<sup>+</sup>), 623 ([*M* + Na]<sup>+</sup>).

Pd/C (10%, 220 mg) was added to a soln. of the tribenzyloxy derivative (733 mg, 1.22 mmol) in MeOH (20 ml). The suspension was stirred under H<sub>2</sub> for 12 h. After filtration, the MeOH was evaporated *in vacuo*. The resulting product was dissolved in H<sub>2</sub>O and lyophilized to provide a white powder (134 mg, 0.50 mmol, 55%; storage under N<sub>2</sub>). M.p. 151.0–152.0°. UV/VIS (EtOH): λ<sub>max</sub> 276.4 (6766). IR (KBr): 3330 (O–H), 1672 (C=O), 1038 (C–O, phenols). <sup>1</sup>H-NMR (D<sub>2</sub>O): 3.70 (s, 3 CH<sub>2</sub>OH); 3.98 (s, CH<sub>2</sub>NH); 6.87 (s, 2 arom. H). <sup>13</sup>C-NMR (D<sub>2</sub>O): 44.61 (C); 61.69 (CH<sub>2</sub>OH); 63.33 (CH<sub>2</sub>NH); 109.06 (*o*-C); 125.74 (CCO); 138.06 (*p*-C); 146.16 (*m*-C); 171.72 (CO); 172.92 (CONH).

N'-[Bis(β-D-galactopyranosyloxy)methyl]([[(nonylamino)carbonyl]oxy)methyl]methyl]-N-(3',4',5'-trihydroxybenzoyl)glycinamide (**5**). BOP (340 mg, 0.77 mmol, 1.2 equiv.) and DMAP (cat. amount) were added to a soln. of N-bis[2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyloxy]methyl]([[(nonylamidocarbonyl)oxy]methyl]methyl]glycinamide [22] (650 mg, 0.64 mmol, 1 equiv.) and of 3,4,5-tribenzyloxybenzoic acid (340 mg, 0.77 mmol, 1.2 equiv.) in CH<sub>2</sub>Cl<sub>2</sub> (50 ml). After stirring for 12 h, the solvent was evaporated under reduced pressure. The resulting crude oil was chromatographed (silica gel; AcOEt/hexane 6:4) to yield the protected intermediate (543 mg, 0.38 mmol, 59%). M.p. 79.0–80.0°. UV/VIS (EtOH): λ<sub>max</sub> 275.0 (6262). IR (KBr): 1751 (C=O, Ac), 1230 (C–O, Ac), 1078 (C–O, galactose). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 0.85 (*t*, *J* = 6.40, Me); 1.23 (s, 6 CH<sub>2</sub>); 1.43 (*m*, NHCH<sub>2</sub>CH<sub>2</sub>); 2.06 (4s, 8 MeCO); 3.05 (*q*, *J* = 6.00, NHCH<sub>2</sub>CH<sub>2</sub>); 3.94 (*m*, 2 CH(6) (galactose), 2 NHCH<sub>2</sub>CO); 4.14 (*m*, 6 H, CH(5) (galactose)); 4.45 (*m*, 2 CH(1) (galactose)); 5.12 (*m*, 2 CH(2), 2 CH(3) (galactose), 2 CH<sub>2</sub>O); 5.40 (s, CH(4) (galactose)); 6.94 (s, NHC (quat.)); 7.05 (*t*, NH(gly)); 7.38 (*m*, 18 H, *o*-CH, arom. H, OCONHCH<sub>2</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>): 14.77 (Me); 21.22–21.46 (MeCO); 23.32–32.52 (CH<sub>2</sub>); 42.00 (NHCH<sub>2</sub>CH<sub>2</sub>); 49.10 (C (quat.)); 61.82 (3 OCH<sub>2</sub>C (quat.)); 67.53–72.01 (C(2), C(3), C(4), C(5) (galactose), *m*-CH<sub>2</sub>O); 75.86 (*p*-CH<sub>2</sub>O); 102.00 (C(1) (β)); 108.04 (*o*-C); 128.23–129.23 (CONH, arom. C); 137.26 (CCH<sub>2</sub>O); 138.00 (*p*-C); 153.54 (*m*-C); 165.40 (OCONHCH<sub>2</sub>); 166.50 (CONH (gly)); 170.88 (CH<sub>2</sub>CONH, MeCOO). FAB<sup>+</sup>-MS: 1430 ([*M* + H]<sup>+</sup>), ([*M* + Na]<sup>+</sup>).

A cat. amount of MeONa was dissolved with the previous intermediate (543 mg, 0.38 mmol) in dist. MeOH (40 ml). The mixture was stirred at r.t. and the reaction monitored by TLC. After completion, the soln. was acidified with an IRC50<sup>®</sup> resin. The resin and solvent were removed to give the deacetylated derivative (410 mg, 0.38 mmol). <sup>1</sup>H-NMR in CD<sub>3</sub>OD confirmed the loss of Ac groups.

Pd/C (10%, 70 mg) was added to a soln. of the tribenzyloxy derivative (410 mg, 0.38 mmol) in MeOH (20 ml). The resulting suspension was stirred for 12 h under H<sub>2</sub>. The Pd was then filtered off, and the MeOH was evaporated. After lyophilization, the expected product **5** (190 mg, 0.23 mmol, 55%) was isolated. M.p. 151.0–152.0°. UV/VIS (EtOH): λ<sub>max</sub> 277.0 (5697). IR (KBr): 3408 (O–H), 1076 (C–O, galactose). <sup>1</sup>H-NMR ((D<sub>6</sub>)DMSO): 0.87 (*t*, *J* = 6.60, Me); 1.25 (s, 6 CH<sub>2</sub>); 1.38 (*m*, NHCH<sub>2</sub>CH<sub>2</sub>); 2.92 (*m*, NHCH<sub>2</sub>); 3.35 (*m*, CH<sub>2</sub>CONH, 2 CH(4 and 5) (galactose), *o*-CH<sub>2</sub>C (quat.), 2 CH<sub>2</sub>(Gal)); 4.12 (*d*, CH(1) (galactose)); 6.85 (s, 2 H, *o*-C); 7.15 (*t*, 1 H, NH(gly)); 7.39 (s, NHC (quat.)); 8.17 (*t*, OCONHCH<sub>2</sub>). <sup>13</sup>C-NMR ((D<sub>6</sub>)DMSO): 13.89 (Me); 22.02 to 28.91 (CH<sub>2</sub>); 31.23 (NHCH<sub>2</sub>CH<sub>2</sub>); 48.53 (C (quat.)); 59.00 (CH<sub>2</sub>O (gal)); 60.30

(CH<sub>2</sub>OCO); 68.08–75.19 (C(4), C(2), C(3), C(5) galactose); 104.52 (C(1) (β)); 106.80 (*o*-C); 124.17 (CCONH); 136.52 (*p*-C); 145.50 (*m*-C); 166.59 (OCONHCH<sub>2</sub>); 169.36 (CH<sub>2</sub>CONH, CONH (gly)).

*N*-(3'',4'',5''-Trihydroxybenzoyl)-*N'*-(tris[(β-D-galactopyranosyl)oxy]methyl)methyl]glycinamide (**6**). *N*-[tris[(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl)oxymethyl]methyl]glycinamide [23] (1.8 g, 1.54 mmol, 1 equiv.) and 3,4,5-tribenzyloxybenzoic acid (815 mg, 1.85 mmol, 1.2 equiv.) were dissolved with BOP (820 mg, 1.85 mmol, 1.2 equiv.) and a cat. amount of DMAP in CH<sub>2</sub>Cl<sub>2</sub> (50 ml). The mixture was stirred at r.t. for 12 h. The resulting soln. was washed 3 × successively with 1N HCl (50 ml), aq. soln. of NaHCO<sub>3</sub> (50 ml), and 2 × with H<sub>2</sub>O. After purification on a silica-gel column (AcOEt/hexane 75:25), the protected intermediate (1.28 g, 0.81 mmol, 53%) was obtained. M.p. 96.0–97.0°. UV/VIS (EtOH): λ<sub>max</sub> 275.0 (6914). IR (KBr): 1078 (C–O, galactose) 1755 (C=O, Ac), 1225 (C–O, galactose). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 2.10 (4s, 12 MeCO); 3.81–3.95 (*m*, 8 H, CH(6) (galactose), CH<sub>2</sub>CO); 4.17 (*m*, 3 CH(5) (galactose), 3 CH<sub>2</sub>O (gal)); 4.44 (*d*, 3 CH(β) (galactose)); 5.04 (*dd*, 3 H, CH(2) (galactose)); 5.10 (*m*, 3 CH(3) (galactose), 3 CH<sub>2</sub>O); 5.40 (*d*, 3 CH(4) (galactose)); 6.27 (s, NHC (quart.)); 7.02 (*t*, NH (gly)); 7.38 (*m*, 17 H, *o*-CH, arom. CH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>): 21.22–21.50 (MeCO); 44.10 (C (quart.)); 60.01–61.81 (CH<sub>2</sub>NH, C(6) (galactose), CH<sub>2</sub>O(Gal)); 67.64–72.05 (*m*-CH<sub>2</sub>O, C(2–5) (galactose)); 75.82 (*p*-CH<sub>2</sub>O); 102.06 (C(1) (β) (galactose)); 107.76 (*o*-C), 128.25–129.82 (CCO (gallic acid), arom. C); 137.40 (CCH<sub>2</sub>O); 139.00 (*p*-C); 153.48 (*m*-C); 167.45 (CO); 169.42–170.68 (CH<sub>2</sub>CONH, MeCOO). FAB<sup>+</sup>-MS: 1591 ([*M*+H]<sup>+</sup>), 1613 ([*M*+Na]<sup>+</sup>). Anal. calc.: C 57.35, H 5.70, N 1.76, found: C 56.27, H 5.72, N 1.97.

The Ac groups of the protected intermediate (745 mg, 0.47 mmol) were removed with a cat. amount of MeONa in MeOH according to the procedure described for **5**. The tribenzyloxy intermediate was obtained (500 mg, 0.46 mmol, 98%). M.p. 160.0–170.0° (dec.). IR (KBr): 1076 (C–O, galactose), 3384 (C–O, galactose). <sup>13</sup>C-NMR (CD<sub>3</sub>OD): 44.50 (C (quat.)); 50.26 (C(6) (galactose)); 61.36 (CH<sub>2</sub>O(Gal)); 62.47 (CH<sub>2</sub>CO); 70.31 (C(4) (galactose)); 72.12 (*m*-CH<sub>2</sub>O); 72.73 (C(2) (galactose)); 75.00 (C(3) (galactose)); 76.20 (*p*-CH<sub>2</sub>O); 76.69 (C(5) (galactose)); 105.49 (C(1) (β)); 107.71 (*o*-C); 128.83–129.64 (CCO, arom. C); 138.40 (CCH<sub>2</sub>); 138.90 (*p*-C); 153.84 (*m*-C); 167.20 (CO); 170.50 (CH<sub>2</sub>CO).

Cleavage of PhCH<sub>2</sub>O groups was performed as described for **5**. The tribenzyloxy derivative (500 mg, 0.46 mmol) was converted to **6** (200 mg, 0.25 mmol, 54%). M.p. ca. 80° (dec.). UV/VIS (EtOH): λ<sub>max</sub> 279.4 (1263). IR (KBr): 1076 (C–O, galactose), 3396 (O–H). <sup>1</sup>H-NMR: in accordance with the structure. <sup>13</sup>C-NMR (D<sub>2</sub>O): 44.89 (C (quat.)); 50.26 (C(6) (galactose)); 61.35 (CH<sub>2</sub>O(Gal)); 62.34 (CH<sub>2</sub>CO); 70–76.52 (C(4), C(2), C(3), C(5) (galactose)); 104.93 (C(1) (β)); 109.17 (*o*-C); 125.80 (CCO (gallic acid)); 138.16 (*p*-C); 146.24 (*m*-C); 171.57 (CO); 172.69 (CH<sub>2</sub>CO).

*Pentafluorophenyl 3,4,5-Tribenzyloxybenzoate*. 3,4,5-Tribenzyloxybenzoic acid (1 g, 2.27 mmol, 1 equiv.), pentafluorophenol (440 mg, 2.50 mmol, 1.1 equiv.), and DCC (500 mg, 2.50 mmol, 1.1 equiv.) were solubilized in freshly distilled CH<sub>2</sub>Cl<sub>2</sub>. The soln. was stirred at r.t. for 4 h. The urea precipitate was filtered off under suction, and the solvent was evaporated. After recrystallization (AcOEt/hexane), a white powder was isolated (1.24 g, 2.05 mmol, 92%). M.p. 124.0–124.5°. UV/VIS (MeOH): λ<sub>max</sub> 280.8 (7746). IR (KBr): 1761 (C=O), 1132 (C–O, ether), 1182 (C–O, ester). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 5.16 (s, 3 CH<sub>2</sub>O); 7.39 (*m*, 15 arom. H), 7.41 (s, 2 H<sub>o</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>): 72.17 (*m*-CH<sub>2</sub>O); 75.97 (*p*-CH<sub>2</sub>O); 111.00 (*o*-C); 122.28 (CCOO); 128.31 (arom. C); 128.78–129.30 (arom. C); 136.98 (CCOO, CCH<sub>2</sub>O); 144.85 (*p*-C); 153.56 (*m*-C); 163.08 (CO).

*N,N'*-(2-Hydroxypropane-1,3-diyl)bis[3,4,5-trihydroxybenzamide] (**7**). Et<sub>3</sub>N (0.16 ml, 1.60 mmol, 2.2 equiv.) was added to 1,3-diaminopropan-2-ol (65 mg, 0.72 mmol, 1 equiv.) dissolved in distilled CH<sub>2</sub>Cl<sub>2</sub> (20 ml). A soln. of pentafluorophenyl 3,4,5-tribenzyloxybenzoate (1 g, 1.60 mmol, 2.2 equiv.) in CH<sub>2</sub>Cl<sub>2</sub> (20 ml) was added dropwise under cooling and N<sub>2</sub>. The soln. was then stirred at r.t. for 12 h. The org. layer was washed with aq. soln. of NaHCO<sub>3</sub> (20 ml). The solvent was removed under reduced pressure, and the resulting crude oil was purified on a silica-gel column (AcOEt/hexane 8:2). After recrystallization, white crystals (200 mg, 0.21 mmol, 30%) were obtained. M.p. 216.0–216.5°. UV/VIS (MeOH): λ<sub>max</sub> 273.4 (5174). IR (KBr): 3288 (O–H), 1630 (C=O), 1130 (C–O). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 3.58 (*m*, 2 CH<sub>2</sub>NH); 3.98 (*m*, *J* = 5.00, HOCH); 4.17 (*d*, *J* = 5, OH); 5.07 (s, 2 *p*-CH<sub>2</sub>O); 5.09 (s, 4 *m*-CH<sub>2</sub>O); 7.37 (*m*, 36 H, *o*-CH, arom. H, CONH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>): 48.36 (HOC); 49.08 (CH<sub>2</sub>NH); 72.06 (*m*-CH<sub>2</sub>O); 75.86 (*p*-CH<sub>2</sub>O); 107.65 (*o*-C); 128.27–129.22 (CCONH, arom. C); 137.27 (*p*-C); 138.11 (CCH<sub>2</sub>O); 153.53 (*m*-C); 167.50 (CO).

The PhCH<sub>2</sub>O groups were removed as described for **5**. The tribenzyloxy intermediate (100 mg, 0.11 mmol) was converted to **7** (25 mg, 0.06 mmol, 55%). M.p. ca. 150° (dec.). UV/VIS (MeOH): λ<sub>max</sub> 276.6 (8146). IR (KBr): 3388 (O–H), 1700 (C=O), 1033 (C–O, phenols). <sup>1</sup>H-NMR ((D<sub>6</sub>)DMSO): 3.24 (*t*, 2 CH<sub>2</sub>NH); 3.72 (*m*, HOCH); 6.86 (s, 4 *o*-CH); 8.05 (*t*, 2 CONH); 8.69 (s, 2 *p*-OH); 9.05 (s, 4 *m*-OH). <sup>13</sup>C-NMR ((D<sub>6</sub>)DMSO): 43.13 (HOC); 56.00 (CH<sub>2</sub>NH); 106.71 (*o*-CH); 124.72 (CCO); 136.24 (*p*-C); 145.41 (*m*-C); 166.75 (CO).

*Tris*-[2-(3',4',5'-trihydroxybenzamido)ethyl]amine (**8**) [31]. The pH of a soln. containing tris(2-aminoethyl)amine (100 mg, 0.68 mmol, 1 equiv.) and pyridine (30 ml) was adjusted to 8–9 by adding a proper amount of DABCO. Pentafluorophenyl 3,4,5-tribenzyloxybenzoate (1.36 g, 2.24 mmol, 3.3 equiv.) was then added at r.t. and stirred for 12 h. Pyridine was removed *in vacuo*, and the crude oil was extracted with AcOEt (3 × 30 ml). This org. phase was washed with H<sub>2</sub>O (2 × 30 ml). Purification was achieved on a silica-gel column (AcOEt) to yield the expected product (480 mg, 0.34 mmol, 50%). M.p. 184–185°. UV/VIS (MeOH):  $\lambda_{\text{max}}$  271.8 (17620). IR (KBr): 1628 (C=O), 1117 (C–O). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 3.24 (*m*, 3 CH<sub>2</sub>N); 3.54 (*m*, 3 NHCH<sub>2</sub>CH<sub>2</sub>); 4.88 (*s*, 9 CH<sub>2</sub>O), 7.23 (*m*, 2 arom. H); 8.40 (*s*, 3 CONH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>): 39.91 (CH<sub>2</sub>N); 56.74 (CH<sub>2</sub>CH<sub>2</sub>NH); 71.77 (*p*-CH<sub>2</sub>O); 75.70 (*m*-CH<sub>2</sub>O); 107.43 (*o*-C); 128.27–129.04 (CCO, arom. C); 137.37 (*p*-C); 138.33, 141.97 (CCH<sub>2</sub>O); 153.38 (*m*-C); 168.35 (CO).

The cleavage of PhCH<sub>2</sub>O groups was performed as described for **5**. The reaction was performed in EtOH (20 ml) with a cat. amount of AcOH. Under these conditions, the tribenzyloxy intermediate (200 mg, 0.14 mmol) gave the deprotected final product **8** (54 mg, 0.09 mmol, 65%). Degradation. UV/VIS (MeOH):  $\lambda_{\text{max}}$  275.0 (18130). IR (KBr): 3419 (O–H), 1047 (C–O, phenols). <sup>1</sup>H-NMR (DMSO): 2.65 (*t*, 3 CH<sub>2</sub>N); 3.28 (*m*, 3 CH<sub>2</sub>CH<sub>2</sub>NH); 4.13 (*s*, 9 OH); 6.79 (*s*, 6 CH<sub>o</sub>); 7.98 (*t*, 3 CONH). Spectroscopic data are in accordance with [31].

*Cotelomers (9)*. The acetylated cotelomers [24] (300 mg) were dissolved in distilled pyridine with pentafluorophenyl 3,4,5-tribenzyloxybenzoate (250 mg, 1 equiv. per free amine belonging to the cotelomer). DABCO was added to the soln. to maintain alkaline conditions. After 16 h at r.t., AcOH (20 ml) was introduced, and the mixture was stirred for an additional 3 h. This soln. was then poured into a large amount of H<sub>2</sub>O. The product was extracted with AcOEt (3 × 30 ml), and the org. layer was washed successively with 1N HCl (3 × 20 ml), sat. aq. NaHCO<sub>3</sub> (20 ml), and H<sub>2</sub>O (20 ml). Purification was achieved on *Sephadex LH20* (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 80:20) to give the functionalized cotelomer (350 mg, 64%). According to the weight of the cotelomers, *i.e.*, the average degree of polymerization or DP<sub>n</sub>, previously specified [24], the number of galloyl unit grafted on the cotelomer backbone was determined by a ratio involving the following integrations: area of benzylic CH<sub>2</sub> signal at 5.05 ppm/area of MeO Lys(ester) signal at 3.73 ppm. This computation gives 13 galloyl units per cotelomer, which means that all free amines are functionalized.

Cleavage of Ac groups was achieved according to the procedure described for **5**. PhCH<sub>2</sub>O groups were removed according to the procedure described for **8**. Cotelomer **9** was isolated (240 mg). IR (KBr): *ca.* 3400 (O–H). <sup>1</sup>H-NMR Spectroscopy in DMSO confirmed the absence of Ac and PhCH<sub>2</sub> groups.

*Antioxidant-Activity Measurements. Oil-in-H<sub>2</sub>O Emulsion* (adapted from [5]). For instance, the solns. of gallic acid was prepared in abs. EtOH at a conc. of 88 mM (soln. *A*) and are tested at 3.5 mM. Another soln. of  $\beta$ -carotene at 200  $\mu$ g/ml, 0.37 · 10<sup>–3</sup> M (soln. *B*) in CHCl<sub>3</sub> was also prepared (the conc. in test tubes was 4  $\mu$ g/ml, 7.4  $\mu$ M).

Soln. *B* (1 ml) was introduced in a 100-ml flask containing 200 mg of *Tween 40* (*Fluka*). CHCl<sub>3</sub> was removed under N<sub>2</sub>, then 20 mg (0.071 · 10<sup>–3</sup> mol) of linoleic acid (*Fluka*) and 50 ml of dist. H<sub>2</sub>O were added to obtain after shaking a micellar soln. (soln. *C*).

Soln. *C* (5 ml) was mixed with 0.2 ml of soln. *A* in screw-capped test vials to obtain a sample, which was studied by spectroscopy at  $\lambda$  464 nm. The reference was made of 5 ml of soln. *C* and 0.2 ml of abs. EtOH. The samples were dipped in a water bath at 50°, and the state of oxidation was measured every 15 min for 8 h.

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