Synthesis of Novel 3,7-Substituted-2-(3′,4′-dihydroxyphenyl)flavones with Improved Antioxidant Activity

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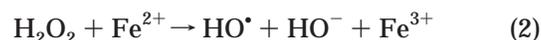
A series of 3,7-disubstituted-2-(3′,4′-dihydroxyphenyl)flavones was synthesized as potential cardioprotective agents in doxorubicin antitumor therapy. The influence of substituents on the 3 and 7 positions of the flavone nucleus on radical scavenging and antioxidant properties was explored to improve the antioxidant activity of our lead compound monoHER. In the TEAC assay most compounds had a similar potency (3.5−5 times as potent as trolox), but in the LPO assay IC$_{50}$ values ranged from 0.2 to 37 μM. In general, the 3-substituted flavones (**9a**−**j**) were the most potent compounds in the LPO assay. The number of hydroxyl groups is not the only prerequisite for antioxidant activity. Substitution in ring A of the flavonoid is not necessary for high activity, but the presence of a 7-OH group significantly modifies the antioxidant activity. The compounds are good antioxidants, which makes it interesting to evaluate them as cardioprotective agents.

Introduction

Flavonoids form a class of benzo-γ-pyrone derivatives which are ubiquitous in plants. The immediate family members of flavonoids include flavones, flavanes, flavonols, anthocyanidins, and catechins. They possess a wide spectrum of biological activities. Some flavonoids have been found to possess anticancer,[1,2] antiischemic,[3] antiallergic, antiinflammatory,[4−6] and several other activities. Recent interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activity of these polyphenolic compounds. These are the result of their high propensity to transfer electrons, to chelate ferrous ions, and to scavenge reactive oxygen species.[7] Because of these properties, flavonoids have been considered as potential protectors against chronic cardiotoxicity caused by the cytostatic drug doxorubicin.

Doxorubicin is a very effective antitumor agent, but its clinical use is limited by the occurrence of a cumulative dose-related cardiotoxicity, resulting in, for example, congestive heart failure. Although the mechanism causing this chronic cardiotoxicity has not been fully elucidated, it is generally believed that the formation of oxygen free radicals plays a crucial role (for reviews, see refs 8−12). In the presence of traces of iron, doxorubicin facilitates the formation of radicals. Moreover, the iron-catalyzed Haber−Weiss reaction (eq 1) or the Fenton reaction (eq 2) is induced, leading to the formation of the extremely reactive hydroxyl radical.

Recently, our group has demonstrated that 7-monohydroxyethylrutoside (monoHER; Chart 1), a semisynthetic flavonoid belonging to a class of hydroxyethylrutosides, provides dose-dependent protection against the doxorubicin-induced cardiotoxicity.[13]

$$H_2O_2 + O_2^{\bullet-} + Fe^{2+} \rightarrow HO^{\bullet} + HO^- + O_2 + Fe^{2+} \quad (1)$$

$$H_2O_2 + Fe^{2+} \rightarrow HO^{\bullet} + HO^- + Fe^{3+} \quad (2)$$

In the present study monoHER has been used as a lead structure for the development of new flavones with improved antioxidant activity. The general structure of the new compounds consists of a flavone backbone with a C2−C3 double bond and a catechol moiety on ring B. According to previously published structure−activity relationships (SARs) of commercially available flavonoids, incorporation of these structural elements leads to potent antioxidants.[14−18] All new compounds were tested for their antioxidant activity in two different assays. First by the trolox equivalent antioxidant capacity assay (TEAC) which is a chemical assay that is a one-phase system containing 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) radicals (ABTS). This assay allows us to study the radical scavenging activity directly without interference by other factors. In addition to this chemical assay, the compounds were tested in a lipid peroxidation assay using rat liver microsomes. In this two-phase system with a lipid and an aqueous phase, not only radical scavenging but, for example, also iron chelation and lipophilicity of the compounds play a role. By comparing the results of these two assays, the influence of the iron chelation and lipophilicity on the antioxidant activity can be estimated.

Previously SAR studies have been performed using either commercially available flavonoids or flavonoids

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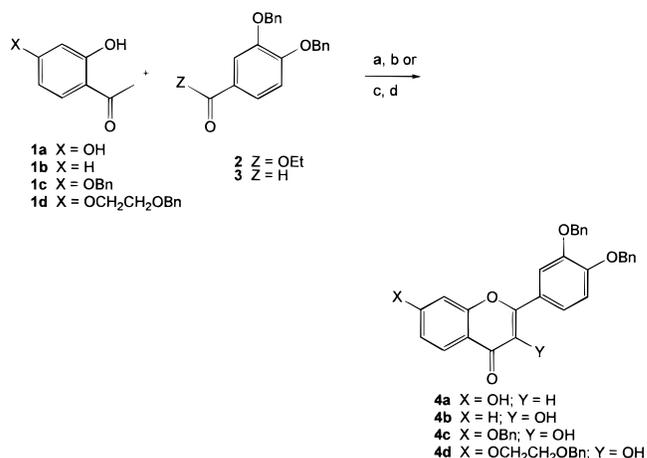**Chart 1.** Chemical Structure of Monohydroxyethylrutoside (monoHER)
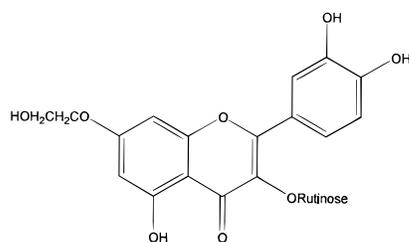
or mixtures thereof isolated from plants.[14,16,17,19−21] These sets of compounds form a structurally highly diverse group of compounds, and consequently it has not been possible to obtain a detailed SAR. In the present study we have focused on the 3,7-disubstituted-2-(3′,4′-dihydroxyphenyl)flavones and studied the influence of the 3 and 7 substituents on the antioxidant activity in a more systematic way. To obtain compounds with an improved cardioprotective profile in vivo compared to monoHER, we have selected substituents that not only modify antioxidant activity but also improve water solubility and cardioselectivity. In 1991, Petty and Grisar prepared an α-tocopherol analogue with improved cardioselectivity by the introduction of a quaternary ammonium group at the 2 position of α-tocopherol.[22,23] We decided to apply this approach and prepared some compounds with a quaternary ammonium group at the 3 or 7 position. The effect of the different substituents at the 3 and 7 positions on the antioxidant profile was evaluated with the TEAC and LPO assays.

Chemistry

The substituted flavonoids were obtained via the four- or five-step synthesis route shown in Scheme 1. Appropriately protected hydroxyacetophenones were coupled to 3,4-dibenzyloxybenzaldehyde according to Pfister et al.[24] to give the corresponding chalcones. The benzyl group was our group of choice for the protection of the hydroxyl groups because of its stability under various reaction conditions and ease of deprotection. The chalcones were subjected to the Algar−Flynn−Oyamada reaction,[25,26] to give the corresponding flavon-3-ols **4b−d** in moderate yields.

In the next step the free hydroxyl group was reacted with a variety of electrophiles (Scheme 2) to give compounds **6b−7d**. The glycosylated flavonoids **9i,j** were prepared via a silver oxide-assisted coupling of an α-bromoperacetylated mono- or disaccharide.[27] The acetyl protective groups were removed with potassium *tert*-butoxide and methanol prior to debenzylation. In the final step the benzyl protective groups were removed either using hydrochloric acid in glacial acetic acid (method A)[28] or by hydrogenolysis in methanol (method B).[29] Method B is strongly preferred over method A, because of the ease of isolation and the higher yield.

The corresponding flavones were obtained via the Baker−Venkataraman[30,31] procedure starting from the appropriately protected hydroxyacetophenones and ethyl 3,4-dibenzyloxybenzoate to give the corresponding flavones in approximately 50% yield. The subsequent functionalization and deprotection procedures were similar to those for the preparation of the flavonols.

Scheme 1[a]

1a X = OH
1b X = H
1c X = OBn
1d X = OCH₂CH₂OBn

2 Z = OEt
3 Z = H

4a X = OH; Y = H
4b X = H; Y = OH
4c X = OBn; Y = OH
4d X = OCH₂CH₂OBn; Y = OH

[a] (a) LiHMDS, THF; (b) DOWEX, 2-propanol; (c) EtOH, 1,4-dioxane, 40% w/v KOH; (d) 1,4-dioxane, EtOH, 5.4% w/v NaOH, 35% H_2O_2.

Biological Evaluation

All compounds were tested in a lipid peroxidation assay to determine their inhibitory effect on the oxidative degradation of membrane lipids. Attack by reactive oxygen species (ROS) produces a lipid peroxy radical, which can either abstract a hydrogen atom from an adjacent lipid to form a lipid hydroperoxide or form a lipid endoperoxide. The formation of lipid endoperoxides in unsaturated fatty acids with at least three double bonds separated by methylene groups leads to the formation of malondialdehyde (MDA) as a breakdown product. In vitro the formed MDA is detected as the pink-colored thiobarbituric acid (TBA) adduct ($\lambda_{max} = 535$ nm).
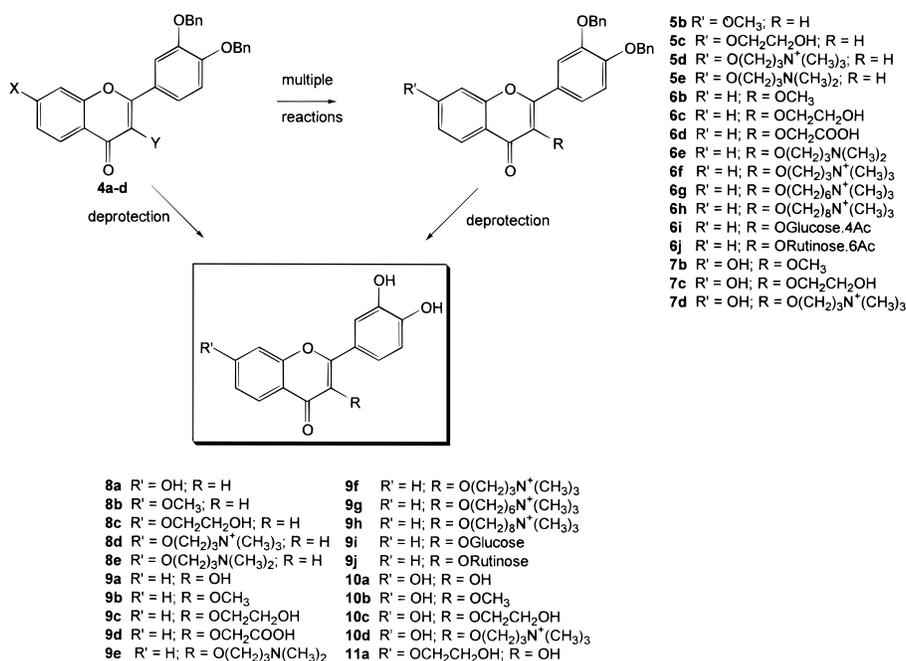
The compounds were also tested in a chemical radical scavenging assay (TEAC assay). This assay is based on the generation and detection of a colored long-lived specific radical cation; ABTS is oxidized by azobis-(amidinepropane) (ABAP) to give the relatively stable ABTS radical. The concentration of ABTS•+ is measured at 734 nm. Thus the antioxidant-induced reduction of the ABTS•+ concentration is directly related to the antioxidant capacity of the compound being tested.

Results and Discussion

Table 1 displays the lipid peroxidation (LPO) and radical scavenging (TEAC) data for compounds **8a−11a**. As can be seen from this table, the compounds show a completely different SAR profile for radical scavenging and lipid peroxidation. In the chemical TEAC assay most compounds have a similar potency (approximately 3.5−5 times more potent than trolox). However, in the LPO assay the IC₅₀ values range from 0.2 to 37.3 μM. The differences between these two assays result from the different experimental circumstances.

In the TEAC assay the radical scavenging activity is measured in an aqueous environment. Therefore, the antioxidant capacity is only dependent on the amount of radicals that can be scavenged by the flavonoid. However, the LPO assay consists of a two-phase system. The combination of Fe^{2+}, vitamin C, and oxygen present in the aqueous phase causes the formation of reactive

Scheme 2

5b R' = OCH$_3$; R = H
5c R' = OCH$_2$CH$_2$OH; R = H
5d R' = O(CH$_2$)$_3$N$^+$(CH$_3$)$_3$; R = H
5e R' = O(CH$_2$)$_3$N(CH$_3$)$_2$; R = H
6b R' = H; R = OCH$_3$
6c R' = H; R = OCH$_2$CH$_2$OH
6d R' = H; R = OCH$_2$COOH
6e R' = H; R = O(CH$_2$)$_3$N(CH$_3$)$_2$
6f R' = H; R = O(CH$_2$)$_3$N$^+$(CH$_3$)$_3$
6g R' = H; R = O(CH$_2$)$_6$N$^+$(CH$_3$)$_3$
6h R' = H; R = O(CH$_2$)$_8$N$^+$(CH$_3$)$_3$
6i R' = H; R = OGlucose.4Ac
6j R' = H; R = ORutinose.6Ac
7b R' = OH; R = OCH$_3$
7c R' = OH; R = OCH$_2$CH$_2$OH
7d R' = OH; R = O(CH$_2$)$_3$N$^+$(CH$_3$)$_3$

8a R' = OH; R = H
8b R' = OCH$_3$; R = H
8c R' = OCH$_2$CH$_2$OH; R = H
8d R' = O(CH$_2$)$_3$N$^+$(CH$_3$)$_3$; R = H
8e R' = O(CH$_2$)$_3$N(CH$_3$)$_2$; R = H
9a R' = H; R = OH
9b R' = H; R = OCH$_3$
9c R' = H; R = OCH$_2$CH$_2$OH
9d R' = H; R = OCH$_2$COOH
9e R' = H; R = O(CH$_2$)$_3$N(CH$_3$)$_2$

9f R' = H; R = O(CH$_2$)$_3$N$^+$(CH$_3$)$_3$
9g R' = H; R = O(CH$_2$)$_6$N$^+$(CH$_3$)$_3$
9h R' = H; R = O(CH$_2$)$_8$N$^+$(CH$_3$)$_3$
9i R' = H; R = OGlucose
9j R' = H; R = ORutinose
10a R' = OH; R = OH
10b R' = OH; R = OCH$_3$
10c R' = OH; R = OCH$_2$CH$_2$OH
10d R' = OH; R = O(CH$_2$)$_3$N$^+$(CH$_3$)$_3$
11a R' = OCH$_2$CH$_2$OH; R = OH

oxygen species (ROS). The resulting degradation of membrane lipids by these ROS is quantified via the amount of MDA–TBA complex formed. This renders the LPO assay a very complex system.

From the results of the TEAC assay we conclude that all listed compounds have a similar radical scavenging capacity. The catechol moiety and the flavone backbone, the two structural elements present in all our compounds, are clearly the functional groups involved in radical scavenging. Compound **9d** has a significantly higher radical scavenging activity than the others. The favorable interaction between the negatively charged substituent and the positively charged ABTS radical probably leads to an overestimation of the radical scavenging capacity of this compound.

The differences in potency of the compounds in the LPO assay are most probably the result of additional properties such as iron chelating ability and lipophilicity. When comparing the LPO IC$_{50}$ values of the new compounds with monoHER, it is clear that almost all new compounds are more potent in protecting against lipid peroxidation. This is in good agreement with previous findings that a catechol moiety in ring B, the 2,3-double bond, and the 4-oxo function are the dominant factors for antioxidant activity.[14-18]

To study the influence of a substituent at the 7 position, we have synthesized a series of compounds with different groups at this position (**8a–e**). Substitution of the 7-hydroxyl group (**8a**) by other groups (**8b–e**) showed that the methoxy-substituted flavone (**8b**) was the most active compound of this series. The high activity of this compound is surprising as it has been reported that a (substituted) 3-hydroxyl group is required for high activity.[15-17] Moreover, this finding is also in sharp contrast with the findings of Cao[19] and Arora[32] who stated that O-methylation of the hydroxyl substituents suppresses the antioxidant activity of the flavonoids. The introduction of a charged quaternary ammonium group at the 7 position (**8d**) is highly unfavorable.

In general, substitution on the 3 position leads to very potent compounds, which are more potent than the 7-substituted flavones. However, the introduction of an acidic moiety (**9d**) at the 3 position causes a 10-fold decrease in the activity in the LPO assay. Under physiological conditions this acidic group is deprotonated and this negative charge is expected to decrease the ability to penetrate the membrane.

The introduction of a quaternary ammonium group at the 3 position (**9f–h**) does not influence the antioxidant activity. Apparently, introduction of a positively charged substituent at C3 is allowed. As a result of the positive charge these compounds dissolve well in water. The fact that these compounds still have potent antioxidant activity in the LPO assay suggests that they also can be found in the membrane. The charged amino group combined with the flavonoid backbone probably allows these compounds to act as amphiphilic compounds, such as the phosphatidylcholines, and this might explain their good antioxidant activity.

The introduction of a sugar moiety at the 3 position is less well tolerated. If the 3-hydroxyethyl group (**9c**) is replaced by a glucose (**9i**) or a rutinose (**9j**) moiety, the activity decreases 5- and 26-fold, respectively. The introduction of a bulky substituent at the 3 position induces an increase in the torsion angle between ring B and the rest of the molecule. This results in a loss of conjugation and a decrease in activity.[33] Furthermore, the introduction of a sugar moiety will also change the lipophilicity and this might affect membrane penetration.

Surprisingly, and in contrast to other reports,[17,32,33] a hydroxyl substituent at the 7 position in combination with a substituent at the 3 position, although of no influence on the radical scavenging capacity, decreases the antioxidant properties (compounds **10a–d**). An exception is the 3-hydroxy compound (**10a**) where the addition of a hydroxyl group improves the antioxidant activity.

A small electron-donating group on position 7 in

Table 1. Yields, Melting Points, and Antioxidant Activities in the Lipid Peroxidation Assay and TEAC Assay

compd	substituent			yield[a] (%)	mp (°C)	LPO IC$_{50}$ (μM) (mean ± SEM, $n = 3$–5)	TEAC (mM) (mean ± SD, $n = 3$–5)
	R$_3$	R$_5$	R$_7$				
8a	H	H	OH	82	300 dec	11.0 ± 1.3	4.3 ± 0.2
8b	H	H	OCH$_3$	88	>240	2.5 ± 0.8	3.3 ± 0.2
8c	H	H	OCH$_2$CH$_2$OH	37	206.7−208.7	16.4 ± 1.7	4.3 ± 0.7
8d	H	H	O(CH$_2$)$_3$N$^+$(CH$_3$)$_3$	74	>210	37.3 ± 2.4	1.9 ± 0.3
8e	H	H	O(CH$_2$)$_3$N(CH$_3$)$_2$	59	173.6	8.1 ± 1.1	2.9 ± 0.4
9a	OH	H	H	69	280 dec	1.8 ± 1.0	4.5 ± 0.5
9b	OCH$_3$	H	H	41	198.5−199.7	1.3 ± 0.7	4.4 ± 0.3
9c	OCH$_2$CH$_2$OH	H	H	42	186.2−186.8	0.6 ± 0.1	4.4 ± 0.4
9d	OCH$_2$COOH	H	H	25	253.3−254.9	6.5 ± 1.0	7.6 ± 0.6
9e	O(CH$_2$)$_3$N(CH$_3$)$_2$	H	H	20	98.5−99.8	1.0 ± 0.1	3.4 ± 0.4
9f	O(CH$_2$)$_3$N$^+$(CH$_3$)$_3$	H	H	27	234.4−235.4	1.2 ± 0.3	4.2 ± 0.2
9g	O(CH$_2$)$_6$N$^+$(CH$_3$)$_3$	H	H	33	230.8−232.8	1.4 ± 0.3	4.3 ± 0.6
9h	O(CH$_2$)$_8$N$^+$(CH$_3$)$_3$	H	H	40	196.7−198.5	1.0 ± 0.3	3.3 ± 0.6
9i	O-glucosyl	H	H	75	166.6−168.6	2.8 ± 0.9	4.9 ± 0.3
9j	O-rutinosyl	H	H	44	174 dec	15.7 ± 4.8	4.3 ± 0.3
10a[b]	OH	H	OH			0.2 ± 0	5.6 ± 0.4
10b	OCH$_3$	H	OH	86	281.4	1.6 ± 0.7	5.9 ± 0.7
10c	OCH$_2$CH$_2$OH	H	OH	77	>300	21.7 ± 6.1	3.4 ± 0.2
10d	O(CH$_2$)$_3$N$^+$(CH$_3$)$_3$	H	OH	10	200 dec	3.8 ± 0.1	5.1 ± 0.3
11a	OH	H	OCH$_2$CH$_2$OH	92	300 dec	13.8 ± 4.4	4.9 ± 0.4
monoHER	O-rutinosyl	OH	OCH$_2$CH$_2$OH			12.7 ± 3.7	3.0 ± 0.2[c]

[a] Yield determined from intermediate to product. [b] Fisetin. [c] van den Berg, R.; Haenen, G. R. M. M.; van den Berg, H.; van der Vijgh, W. J. F.; Bast, A. The predictive value of the antioxidant capacity of structurally related flavonoids using the trolox equivalent antioxidant capacity (TEAC) assay. *Food Chem.*, in press.

combination with substituents on the 3 position is allowed for activity; see also compounds **8a,b**.

The influence of the 5-OH group present in monoHER has not been evaluated. These 3,5,7-trisubstituted compounds are synthetically not easily accessible, and the present study shows that substituents at C5 are not required for excellent antioxidant activity.

Conclusions

We have successfully developed a synthesis route for the preparation of 3-substituted, 7-substituted, and 3,7-disubstituted-2-(3′, 4′-dihydroxyphenyl)flavones. All new compounds are potent radical scavengers. In the LPO assay the 3-substituted compounds are superior to the 7-substituted compounds. We found that the catechol moiety in combination with a C2−C3 double bond and a 4-keto function are the essential structural elements for potent antioxidant activity. From previous SAR studies[19,34] it was concluded that the antioxidant activity correlates with the number of phenolic OH groups present. With our series of compounds we have shown that this is not necessarily true, as our 3-substituted flavones without substituents in ring A are the most potent compounds. Introduction of an additional OH on the 7 position in combination with a 3 substituent does not improve activity unless an OH group is present at the 3 position. This finding is in contrast to results obtained from the highly diverse sets of commercially available flavonoids that are usually investigated. Additionally we have shown that substitution of C7 significantly modifies the antioxidant activity of the molecule, in contrast to earlier findings.[17,32]

We successfully prepared new flavonoids which are of interest as potential cardioprotectors; they might be more active than monoHER in vivo.

Experimental Section

Instruments and Analyses. Elemental analyses were performed for C, H, N (Department of Microanalysis, Groningen University, The Netherlands). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 (200 MHz) spectrometer. Chemical shifts for ^{1}H and ^{13}C NMR are given in ppm (δ) relative to tetramethylsilane (TMS) as internal standard. Melting points (not corrected) were measured on an Electrothermal AI-9100 apparatus. THF was dried over LiAlH$_4$ and distilled before use. 4-Benzyloxy-2-hydroxyacetophenone,[35] β-bromoethylbenzyl ether[36] and ethyl 3,4-dibenzyloxybenzoate[37] were prepared according to literature procedures.

3′,4′-Dibenzyloxy-7-hydroxyflavone (4a). 32.28 g (200 mmol) of 1,1,1,3,3,3-hexamethyldisilazan (HMDS) was dissolved in dry THF (250 mL) and cooled to −60 °C under an atmosphere of dry nitrogen. 125 mL (200 mmol) of butyllithium (1.6 M in hexane) was added dropwise followed by 50 mmol of 2,4-dihydroxyacetophenone (**1a**) in dry THF (100 mL) and stirred for 45 min at −30 °C. The reaction mixture was cooled to −60 °C and 19.4 g (50 mmol) of **2** in dry THF (100 mL) was added dropwise. This mixture was stirred for another 45 min at −60 °C and at room temperature overnight. The reaction mixture was poured on ice and acidified with 3.0 M HCl. The THF was removed under reduced pressure and the residue was extracted with chloroform (2 × 150 mL). The chloroform layers were dried over sodium sulfate and concentrated in vacuo. The crude 1-(2,4-dihydroxyphenyl)-3-(3,4-dibenzyloxy-phenyl)-1,3-propanedione was dissolved in 2-propanol (100 mL), 25 g of Dowex W50 × 8 (H$^+$ form) was added and heated under reflux for 16 h under an atmosphere of dry nitrogen. The solids were filtered, suspended in DMF and filtered to remove the Dowex. The filtrate was concentrated under reduced pressure to give 11.0 g (49%) **4a** as a white solid; mp 245.4−245.8 °C. ^{1}H NMR (DMSO): δ 5.25 (s, 2H, OC*H*$_2$Ph), 5.29 (s, 2H, OC*H*$_2$Ph), 6.87 (s, 1H, C3H), 6.91 (dd, 2H, *J* = 9 Hz, 2 Hz, C5′H), 7.0 (d, 1H, *J* = 2 Hz, C8H), 7.21 (d, *J* = 8 Hz, C6H), 7.3−7.6 (m, 10H, OCH$_2$*Ph* × 2), 7.64 (dd, 1H, *J* = 7 Hz, 2 Hz, C6′H), 7.70 (t, *J* = 2 Hz, C2′H), 7.86 (d, 1H, *J* = 9 Hz, C5H), 10.9 (br s, 1H, OH).

3′,4′-Dibenzyloxy-3-hydroxyflavone (4b). A suspension of 31.4 mmol of 3,4-dibenzyloxybenzaldehyde (**3**) and 31.4 mmol of 2-hydroxyacetophenone (**1b**) in ethanol (80 mL) and dioxane (50 mL) was cooled to 10 °C and 25 mL of 40% w/v KOH solution was added dropwise. The reaction mixture was stirred for 66 h at room temperature. CH$_2$Cl$_2$ (400 mL) was added and the organic layer was washed with H$_2$O (3 × 50 mL), dried over sodium sulfate and concentrated in vacuo. The oily residue was dissolved in dioxane (110 mL) and ethanol (300 mL), and 5.4% (w/v) NaOH solution (100 mL). 11.4 mL of 35% H$_2$O$_2$ was added dropwise. The reaction mixture was

stirred in an ice bath for 2 h and subsequently at room temperature overnight, resulting in a yellow suspension. After acidification with 2 M HCl (100 mL) the precipitate was filtered and washed with H_2O (500 mL). The crude product was recrystallized from ethanol to give 7.0 g (49%) of a light yellow solid; mp 145.8–146.8 °C. ^{1}H NMR (CDCl$_3$): δ 5.22 (s, 2H, OCH_2Ph), 5.25 (s, 2H, OCH_2Ph), 7.01 (d, $J = 8$ Hz, C5′H), 7.08 (br s, 1H, C3OH), 7.3–7.5 (m, 12 H, OC$H_2Ph \times 2$ + C6H + C8H), 7.64 (dt, 1H, $J = 7$ Hz, 1.5 Hz, C7H), 7.82 (dd, 1H, $J = 9$ Hz, 2 Hz, C6′H), 7.92 (d, 1H, $J = 2$ Hz, C2′H), 8.20 (dd, 1H, $J = 8$ Hz, 2 Hz, C5H).

3-Hydroxy-7,3′,4′-tribenzyloxyflavone (4c). 3,4-Dibenzyloxybenzaldehyde (**3**) was reacted with 4-benzyloxy-2-hydroxyacetophenone (**1c**) in a similar way as described for **4b**. The brown oil resulting from step 1 was crystallized from ethanol/chloroform (5:1) to give 1.8 g (42%); mp 120.3–121.3 °C. In the second step the product was extracted with CH$_2$Cl$_2$ (2 × 100 mL). The organic layers were dried over sodium sulfate and evaporated under reduced pressure. The product was crystallized form ethanol/CH$_2$Cl$_2$ (100 mL, 2:1) to give 1.82 g (41%) of **4c**; mp 160.6–161.2 °C. ^{1}H NMR (CDCl$_3$): δ 5.16 (s, 2H, C7OCH_2Ph), 5.24 (s, 4H, C3′OCH_2Ph + C4′OCH_2Ph), 6.9 (br s, 1H, C3OH), 6.96–7.04 (m, 3H, C5′H + C6H + C8H), 7.23–7.47 (m, 15H, 3× OCH_2Ph), 7.77 (dd, 1H, $J = 9$ Hz, 2 Hz, C6′H), 7.87 (d, 1H, $J = 2$ Hz, C2′H), 8.10 (d, 1H, $J = 9$ Hz, C5H).

7-(2-Benzyloxyethoxy)-3-hydroxy-3′,4′-dibenzyloxyflavone (4d). A stirred suspension of 20 mmol of 2,4-dihydroxyacetophenone, 20 mmol of β-bromoethylbenzyl ether and 24 mmol of K$_2$CO$_3$ in acetone (60 mL) was heated to reflux for 16 h. The solvent was evaporated and the residue resuspended in H$_2$O (100 mL) and extracted with CHCl$_3$ (2 × 100 mL). The combined layers were dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (CH$_2$Cl$_2$) to give 3.7 g (65%) of a colorless liquid. ^{1}H NMR (CDCl$_3$): δ 2.53 (s, 3H, CH$_3$), 3.81 (t, 2H, $J = 6$ Hz, BnOCH_2CH$_2$O), 4.14 (t, 2H, $J = 6$ Hz, BnOCH$_2$CH_2O), 4.60 (s, 2H, OCH_2Ph), 6.40 (m, 2H, C3H + C5H), 7.33 (s, 5H, OCH$_2Ph$), 7.60 (d, 1H, $J = 9$ Hz, C6H), 12.71 (s, 1H, OH).

3,4-Dibenzyloxybenzaldehyde (**3**) was reacted with 2-hydroxy-4-(2-benzyloxyethoxy)acetophenone (**1d**) in a similar way as described for **4b**. The brown oil resulting from step 1 was crystallized from ethanol/diethyl ether to give 2.0 g (49%). In the second step 70 mL of 0.8% w/v NaOH instead of 100 mL of 5.4% NaOH was used and the product was extracted with CHCl$_3$ (2 × 100 mL). The organic layers were dried over sodiumsulfate and evaporated under reduced pressure. The product was crystallized from methanol/CHCl$_3$ (4:1) to give 1.2 g (59%) of **4d**. ^{1}H NMR (CDCl$_3$): δ 3.87 (t, 2H, $J = 6$ Hz, BnOCH_2CH$_2$O), 4.25 (t, 2H, $J = 6$ Hz, BnOCH$_2$CH_2O), 4.64 (s, 2H, PhCH$_2$–OCH$_2$CH$_2$O), 5.24 (s, 4H, C3′OCH_2Ph + C4′OCH_2Ph), 6.88–7.04 (m, 3H, C8H + C5′H + C6H), 7.24–7.51 (m, 15H, PhOCH$_2$CH$_2$, C3′OCH_2Ph + C4′OCH_2Ph), 7.78 (dd, 1H, $J = 9$ Hz, 2 Hz, C6′H), 7.85 (d, 1H, $J = 2$ Hz, C2′H), 8.05 (d, 1H, $J = 9$ Hz, C5H).

3′,4′-Dibenzyloxy-7-methoxyflavone (5b). 2.2 mmol of potassium *tert*-butoxide was added to a suspension of 2.0 mmol of **4a** in dry THF (50 mL), followed by 8.8 mmol of CH$_3$I and the mixture was stirred at room temperature for 16 h. H$_2$O (100 mL) was added and the mixture was extracted with CH$_2$-Cl$_2$ (2 × 100 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure to give 0.9 g (100%) of **5b** as a white solid. ^{1}H NMR (CDCl$_3$): δ 3.87 (s, 3H, OCH$_3$), 5.19 (s, 4H, OCH_2Ph × 2), 6.55 (s, 1H, C3H), 6.8–7.0 (m, 3H, C5′H + C6H + C8H), 7.26–7.44 (m, 12H, OCH$_2Ph$ × 2 + C2′H +C6′H), 8.04 (d, 1H, $J = 9$ Hz, C5H).

3′4′-Dibenzyloxy-3-methoxyflavone (6b). The reaction was carried out with **4b**, using the same method as described for **5b**. The product was crystallized twice from ethyl acetate and hexane to give 240 mg (47%) as a white solid. ^{1}H NMR (CDCl$_3$): δ 3.74 (s, 3H, OCH$_3$), 5.28 (s, 4H, OCH_2Ph), 6.98 (d, $J = 7$ Hz, C5′H), 7.3–7.7 (m, 14H, OCH$_2Ph$ × 2 + C6H + C8H

+ C7H + C6′H), 7.82 (d, 1H, $J = 2$ Hz, C2′H), 8.23 (dd, 1H, $J = 7$ Hz, 2 Hz, C5H).

3-Methoxy-7,3′,4′-tribenzyloxyflavone (7b). **4c** was reacted as described for **5b**; yield 1.0 g (96%). ^{1}H NMR (CDCl$_3$): δ 3.70 (s, 3H, OCH$_3$), 5.14 (s, 2H, C7OCH_2Ph), 5.25 (s, 4H, C3′OCH_2Ph + C4′OCH_2Ph), 6.90 (d, 1H, $J = 2$ Hz, C8H), 7.0–7.1 (m, 2H, C5′H + C6H), 7.28–7.49 (m, 15H, 3× OCH$_2Ph$), 7.66 (dd, 1H, $J = 7$ Hz, 2 Hz, C6′H), 7.77 (d, 1H, $J = 2$ Hz, C2′H), 8.12 (d, 1H, $J = 9$ Hz, C5H).

3′,4′-Dibenzyloxy-7-(hydroxyethoxy)flavone (5c). A mixture of 2.2 mmol of **4a** and 44 mmol of ethylenecarbonate was heated to 95 °C, 23 mmol of powdered K$_2$CO$_3$ was added and the reaction mixture was stirred at 95 °C for 1 h. Chloroform (50 mL) was added and the K$_2$CO$_3$ was filtered. The filtrate was washed with 5 N HCl (3 × 10 mL), dried over sodium sulfate and concentrated under reduced pressure. The product was recrystallized from ethanol to give 0.5 g (45%) of **5c** as a yellow powder. ^{1}H NMR (DMSO): δ 3.75 (t, 2H, $J = 6$ Hz, OCH_2CH$_2$OH), 4.17 (t, 2H, $J = 6$ Hz, OCH$_2$CH_2OH), 5.23 (s, 2H, C3′OCH_2Ph), 5.29 (s, 2H, C4′OCH_2Ph), 6.92 (s, 1H, C3H), 7.08 (d, 1H, $J = 9$ Hz, 2 Hz, C5′H), 7.25 (d, 1H, $J = 7$ Hz, C8H), 7.3–7.6 (m, 10H, OCH$_2Ph$ × 2), 7.73 (dd, 1H, $J = 9$ Hz, 2 Hz, C6′H), 7.78 (d, 1H, $J = 2$ Hz, C2′H), 7.94 (d, 1H, $J = 7$ Hz, C5H).

3′,4′-Dibenzyloxy-3-(hydroxyethoxy)flavone (6c). The reaction was carried out with β-bromoethylbenzyl ether and **4b**, using the same method as described for **4d** (step 2); yield 240 mg (49%) of **6c** as a light-yellow solid. ^{1}H NMR (CDCl$_3$): δ 3.69 (t, 2H, $J = 6$ Hz, CH_2OCH$_2$Ph), 4.27 (t, 2H, $J = 6$ Hz, C3OCH_2), 4.40 (s, 2H, OCH_2Ph), 5.14 (s, 2H, OCH_2Ph), 5.27 (s, 2H, OCH_2Ph), 6.86 (d, 1H, $J = 9$ Hz, C5′H), 7.2–7.5 (m, 12H, OCH$_2Ph$ × 3 + C6H + C8H), 7.64 (dt, 1H, $J = 7$ Hz, 2 Hz, C7H), 7.8–7.9 (m, 2H, C2′H + C6′H), 8.20 (dd, $J = 7$ Hz, 2 Hz, C5H).

3-Hydroxyethoxy-7,3′,4′-tribenzyloxyflavone (7c). The reaction was carried out with **4c**, using the same method as described for **5c**. The product was recrystallized from ethanol and chloroform to give 1.15 g (96%) of **7c** as a light-yellow solid; mp 136.7 °C. ^{1}H NMR (CDCl$_3$): δ 3.6–3.8 (m, 4H, OCH$_2$CH$_2$O), 5.15 (s, 2H, OCH_2Ph), 5.25 (s, 2H, OCH_2Ph), 5.26 (s, 2H, OCH_2Ph), 6.92 (d, 1H, $J = 2$ Hz, C8H), 7.0–7.1 (m, 2H, C6H + C5′H), 7.2–7.5 (m, 10H, OCH$_2Ph$ × 2), 7.63 (dd, 1H, $J = 8$ Hz, 2 Hz, C6′H), 7.74 (d, 1H, $J = 2$ Hz, C2′H), 8.12 (d, 1H, $J = 9$ Hz, C5H).

N-(3-(3′,4′-Dibenzyloxyflavon-7-yl)oxypropyl)-N,N,N,-trimethylammonium Chloride (5d). To a solution of 6.0 mmol of **4a** in DMSO (30 mL) were added 17.4 mmol of K$_2$-CO$_3$ and 1.2 mL (12.0 mmol) of 1-bromo-3-chloropropane and the mixture was stirred at room temperature for 5 h. The DMSO was evaporated under reduced pressure. The residue was suspended in CH$_2$Cl$_2$ (100 mL), filtered and concentrated in vacuo to give 3.0 g (95%) of 7-(3-chloropropoxy)-3′,4′-dibenzyloxyflavone; mp 152.6–153.4 °C. ^{1}H NMR (CDCl$_3$): δ 2.29 (m, 2H, CCH_2C), 3.76 (t, 2H, $J = 6$ Hz, CH$_2$Cl), 4.22 (t, 2H, $J = 6$ Hz, OCH$_2$), 5.23 (s, 4H, OCH_2Ph × 2), 6.59 (s, 1H, C3H), 6.89–7.02 (m, 3H, C6H + C8H + C5′H), 7.30–7.50 (m, 12H, OCH$_2Ph$ × 2 + C2′H + C6′H), 8.08 (d, 1H, $J = 9$ Hz, C5H).

A mixture of 2.5 mmol of 7-(3-chloropropoxy)-3′,4′-dibenzyloxyflavone, 15 mL of trimethylamine (33% in ethanol) and 2 mmol of K$_2$CO$_3$ in dioxane (15 mL) was heated at 100 °C for 56 h in a stainless steel bomb. The mixture was filtered and the solvents were evaporated under reduced pressure. The product was recrystallized twice from 2-propanol/ethanol to give 1.26 g (86%) of **5d** as a white solid. ^{1}H NMR (DMSO): δ 2.27 (m, 2H, CCH_2C), 3.12 (s, 9H, N(CH$_3$)$_3$), 3.82 (t, 2H, $J = 6$ Hz, CH$_2$N), 4.24 (t, 2H, $J = 6$ Hz, OCH$_2$), 5.24 (s, 2H, C3′OCH_2-Ph), 5.27 (s, 2H, C4′OCH_2Ph), 6.93 (s, 1H, C3H), 7.0–8.0 (m, 16H, C6′H + C2′H + C6H + C8H + C5′H + C3′OCH$_2Ph$ + C4′OCH$_2Ph$ + C5H).

N-(3-(3′,4′-Dibenzyloxyflavon-3-yl)oxypropyl)-N,N,N-trimethylammonium Bromide (6f). 4.5 mL (44.3 mmol) of 1,3-dibromopropane was added to a solution of 11.1 mmol of **4b** and 11.8 mmol of potassium *tert*-butoxide in dry THF (250

mL). The reaction mixture was stirred at room temperature for 19 h and subsequently heated to reflux for 5 h under an atmosphere of dry nitrogen. CH$_2$Cl$_2$ (250 mL) was added and the mixture was washed with H$_2$O. The organic layer was dried over magnesium sulfate and evaporated under reduced pressure. The crude product was purified by column chromatography (ethyl acetate/hexane 1:1 (v/v)) and recrystallized from ethyl acetate and hexane to give 2.71 g (42%) of 3-(3-bromopropoxy)-3′,4′-dibenzyloxyflavone. ^{1}H NMR (CDCl$_3$): δ 218 (m, 2H, CCH_2C), 3.47 (t, 2H, *J* = 6 Hz, CH$_2$Cl), 4.13 (t, 2H, *J* = 6 Hz, OCH$_2$), 5.23 (s, 2H, OCH_2Ph), 5.27 (s, 2H, OCH_2-Ph), 7.04 (s, 1H, C3H), 7.25−7.5 (m, 3H, C6H + C8H + C5′H), 7.6−7.8 (m, 12H, OCH$_2$*Ph* × 2 + C2′H + C6′H), 8.23 (dd, 1H, *J* = 7 Hz, 2 Hz, C5H).

A mixture of 4.7 mmol of 3-(3-bromopropoxy)-3′,4′-dibenzyloxyflavone and 25 mL of trimethylamine (33% in ethanol) in dioxane (15 mL) was heated to 100 °C for 17 h in a stainless steel bomb. The solvents were evaporated under reduced pressure and the product was recrystallized from ethanol and hexane to give 2.1 g (71%) of **6f** as an off-white powder. ^{1}H NMR (DMSO): δ 2.03−2.18 (m, 2H, CH$_2$), 3.38 (s, 9H, N(CH$_3$)$_3$), 3.45−3.55 (m, 2H, CH$_2$N), 4.0 (t, 2H, *J* = 7 Hz, OCH$_2$), 5.24 (s, 2H, OCH_2Ph), 5.28 (s, 2H, OCH_2Ph), 7.26−7.55 (m, 11H, 2× OCH$_2$*Ph* + C5′H), 7.70−7.92 (m, 5H, C6H + C8H + C7H + C6′H + C2′H), 8.10 (d, 1H, *J* = 9 Hz, C5H).

N-(6-(3′,4′-Dibenzyloxyflavon-3-yl)oxyhexyl)-N,N,N-trimethylammonium Chloride (6g). **6g** was prepared by alkylation of **4b** with 1-chloro-6-iodohexane, using the same method as described for **5d**. The crude product was purified by column chromatography (CH$_2$Cl$_2$) to give 1.6 g (94%) of 3-(6-chlorohexyloxy)-3′,4′-dibenzyloxyflavone. ^{1}H NMR (CDCl$_3$): δ 1.36 (m, 4H, CH$_2$), 1.68 (m, 4H, CH$_2$), 3.47 (t, 2H, *J* = 6 Hz, CH$_2$Cl), 3.99 (t, 2H, *J* = 6 Hz, OCH$_2$), 5.23 (s, 2H, OCH_2Ph), 5.27 (s, 2H, OCH_2Ph), 7.03 (d, 1H, *J* = 8 Hz, C5′H), 7.25−7.5 (m, 12H, 2× OCH$_2$*Ph* + C6H, C8H), 7.66 (dt, 1H, *J* = 7 Hz, 2 Hz, C7H), 7.71 (dd, 1H, *J* = 8 Hz, 1 Hz, C6′H), 7.80 (d, 1H, *J* = 1 Hz, C2′H), 8.23 (dd, 1H, *J* = 7 Hz, 2 Hz, C5H).

3-(6-Chlorohexyloxy)-3′,4′-dibenzyloxyflavone was reacted in a similar way as described for **5d,** without the addition of K$_2$CO$_3$. The product was crystallized from ethanol and ether to give 1.14 g (69%) **6g**. ^{1}H NMR (CDCl$_3$): δ 1.2−1.7 (m, 8H, CH$_2$), 3.28 (s, 9H, N$^+$(CH$_3$)$_3$), 3.42 (t, 2H, *J* = 7 Hz, CH$_2$N), 3.86 (t, 2H, *J* = 7 Hz, OCH$_2$), 5.14 (s, 2H, OCH_2Ph), 5.19 (s, 2H, OCH_2-Ph), 7.03 (d, 1H, *J* = 9 Hz, C5′H), 7.10−7.70 (m, 15H, 2× OCH$_2$*Ph* + C6H + C8H + C7H + C6′H + C2′H), 8.13 (dd, 1H, *J* = 9 Hz, C5H).

N-(8-(3′,4′-Dibenzyloxyflavon-3-yl)oxyoctyl)-N,N,N-trimethylammonium Bromide (6h). **6h** was prepared by alkylation of **4b** with 1,8-dibromooctane, using the same method as described for **5d**; yield 1.05 g (82%) of 3-(8-bromooctyloxy)-3′,4′-dibenzyloxyflavone. ^{1}H NMR (CDCl$_3$): δ 1.2−1.5 (m, 8H, O(CH$_2$)$_3$(CH_2)$_4$CH$_2$Br), 1.5−1.9 (m, 4H, OCH$_2$-(CH_2)$_2$(CH$_2$)$_5$Br), 3.34 (t, 2H, *J* = 7 Hz O(CH$_2$)$_7$CH_2Br), 3.97 (t, 2H, *J* = 7 Hz, OCH$_2$(CH$_2$)$_7$Br), 5.22 (s, 2H, C3′OCH_2Ph), 5.25 (s, 2H, C4′OCH_2Ph), 7.02 (d, 1H, *J* = 9 Hz, C5′H), 7.25−7.55 (m, 12H, C3′OCH$_2$*Ph* + C4′OCH$_2$*Ph* + C6H + C8H), 7.59−7.73 (m, 2H, C6′H + C7H), 7.81 (d, 1H, *J* = 2 Hz, C2′H), 8.22 (dd, 1H, *J* = 8.0 Hz, 2 Hz, C5′H).

3-(8-Bromooctyloxy)-3′,4′-dibenzyloxyflavone was reacted in a similar way as described for **6f**, with the modification that 3-(8-bromooctyloxy)-3′,4′-dibenzyloxyflavone was dissolved in ethanol and 1,4-dioxane; yield 0.93 g (91%) of a yellow solid. ^{1}H NMR (CDCl$_3$): δ 1.2−1.7 (m, 12H, CH$_2$), 3.23 (s, 9H, N$^+$-(CH$_3$)$_3$), 3.38 (t, 2H, *J* = 9 Hz, O(CH$_2$)$_7$CH$_2$N$^+$(CH$_3$)$_3$), 3.97 (t, 2H, *J* = 6 Hz, OCH$_2$), 5.18 (s, 2H, C3′OCH_2Ph), 5.21 (s, 2H, C4′OCH_2Ph), 7.07 (d, 1H, *J* = 9 Hz, C5′H), 7.33−7.50 (m, 12H, C3′OCH$_2$*Ph* + C4′OCH$_2$*Ph* + C6H + C8H), 7.61−7.72 (m, 2H, C6′H + C7H), 7.79 (d, 1H, *J* = 2 Hz, C2′H), 8.18 (dd, 1H, *J* = 7 Hz, 2 Hz, C5H).

N-(3-(7,3′,4′-Tribenzyloxyflavon-3-yl)oxypropyl)-N,N,N-trimethylammonium Chloride (7d). **4c** was alkylated using 1-bromo-3-chloropropane, employing the same method as described for **5d**. The product was purified by column chromatography (CH$_2$Cl$_2$) to give 3.2 g (84%) of 3-(3-chloropropoxy)-

7,3′,4′-tribenzyloxyflavone; mp 136.1−137.3 °C. ^{1}H NMR (CDCl$_3$): δ 2.06 (m, 2H, OCH$_2$CH_2CH$_2$Cl), 3.59 (t, 2H, *J* = 7 Hz, OCH$_2$CH$_2$CH_2Cl), 4.08 (t, 2H, *J* = 7 Hz, OCH_2CH$_2$CH$_2$-Cl), 5.15 (s, 2H, C7OCH_2Ph), 5.22 (s, 2H, C3′OCH_2Ph), 5.24 (s, 2H, C4′OCH_2Ph), 6.89 (d, 1H, *J* = 1 Hz, C8H), 7.0−7.1 (m, 2H, C5′H + C6H), 7.3−7.8 (m, 17H, 3× OCH$_2$*Ph* +C2′H + C6′H), 8.12 (d, 1H, *J* = 7 Hz, C5H).

A mixture of 2.0 mmol of 3-(3-chloropropoxy)-7,3′,4′-tribenzyloxyflavone in 15 mL of trimethylamine (33% in ethanol) was heated to 100 °C for 64 h in a stainless steel bomb. The solvents were evaporated under reduced pressure and the product was crystallized from toluene to give 167 mg (12%) of a white solid; mp 174.7−174.8 °C. ^{1}H NMR (DMSO): δ 2.08 (m, 2H, OCH$_2$CH_2CH$_2$N(CH$_3$)$_3$), 3.06 (s, 9H, N$^+$(CH$_3$)$_3$), 3.52 (m, 2H, OCH$_2$CH$_2$CH_2N(CH$_3$)$_3$), 4.08 (t, 2H, *J* = 7 Hz, OCH_2-CH$_2$CH$_2$N(CH$_3$)$_3$), 5.23 (s, 2H, C7OCH_2Ph), 5.27 (s, 4H, 2x OCH_2Ph), 7.14 (dd, 1H, *J* = 9 Hz, 2 Hz, C5′H), 7.20−7.70 (m, 19H, 3× OCH2Ph + C6H + C8H + C6′H + C2′H), 7.98 (d, 1H, *J* = 9 Hz, C5H).

3′,4′-Dibenzyloxy-7-(3-dimethylaminopropoxy)flavone (5e). **4a** was reacted in a similar way as described for **5d** using dimethylamine (33% in ethanol). The residue was suspended in acidic water (pH = 1−2), washed with chloroform (2 × 100 mL), basified with 1 M NaOH and extracted with chloroform (2 × 150 mL). The organic layers were dried over sodium sulfate and concentrated in vacuo to give 0.8 g (60%) of **5e**. ^{1}H NMR (CDCl$_3$ + 20% DMSO): δ 2.37 (m, 2H, CCH_2C), 2.86 (d, 6H, *J* = 7 Hz, N(CH$_3$)$_2$), 3.30 (m, 2H, CH$_2$N), 4.30 (t, 2H, *J* = 6 Hz, OCH$_2$), 5.21 (s, 2H, C3′OCH_2Ph), 5.23 (s, 2H, C4′OCH_2Ph), 6.70 (s, 1H, C3H), 6.98−7.13 (m, 3H, C6H + C8H + C5′H), 7.33−7.56 (m, 12H, OCH$_2$*Ph* × 2 + C2′H + C6′H), 7.98 (d, 1H, *J* = 9 Hz, C5H).

3′,4′-Dibenzyloxy-3-(3-dimethylaminopropoxy)flavone (6e). **4b** was prepared using the method described for **5d**. After evaporation of the DMSO the residue was dissolved in H$_2$O (100 mL) and extracted with chloroform (2 × 100 mL). The organic layers were dried over sodium sulfate and evaporated under reduced pressure to give 1.5 g (95%) of 3-(3-chloropropoxy)-3′,4′-dibenzyloxyflavone. ^{1}H NMR (CDCl$_3$): δ 2.08 (m, 2H, OCH$_2$CH_2CH$_2$Cl), 3.60 (t, 2H, *J* = 7 Hz, OCH$_2$-CH$_2$CH_2Cl), 4.11 (t, 2H, *J* = 7 Hz, OCH_2CH$_2$CH$_2$Cl), 5.26 (s, 4H, C3′OCH_2Ph + C4′OCH_2Ph), 7.12 (d, 1H, *J* = 9 Hz, C5′H), 7.26−7.52 (m, 17H, 3× OCH$_2$*Ph* + C6H + C8H), 7.60−7.70 (m, 3H, C2′H + C6′H + C7H), 8.20 (d, 1H, *J* = 9 Hz, C5H).

A mixture of 2.0 mmol of 3-(3-chloropropoxy)-3′,4′-dibenzyloxyflavone in ethanol (10 mL) and dioxane (10 mL), 2.0 mmol of NaI, 4.0 mmol of Na$_2$CO$_3$ and 25 mL of dimethylamine (33% in ethanol) was heated to 60 °C for 56 h in a stainless steel bomb. The solvents were evaporated under reduced pressure. The resulting brown oil was used directly in the next step.

(3′,4′-Dibenzyloxyflavon-3-yl)oxyacetic Acid (6d). To a stirred solution of 2.0 mmol of **4b** and 4.0 mmol of potassium *tert*-butoxide in dry THF (25 mL) was added 6.1 mmol of ethyl chloroacetate. The reaction mixture was stirred at room temperature for 16 h. After acidification with concentrated HCl, ice-cold H$_2$O (100 mL) was added and the mixture was extracted with CH$_2$Cl$_2$ (4 × 25 mL). The organic layers were dried over magnesium sulfate and evaporated in vacuo. The product was crystallized from ethyl acetate to give 0.62 g (1.15 mmol 57%) of ethyl (3′,4′-dibenzyloxyflavon-3-yl)oxyacetate as an off-white powder.

The powder was dissolved in 1,4-dioxane and a solution of 2.07 mmol of NaOH dissolved in 7.5 mL of methanol was added. The reaction mixture was stirred at room temperature for 16 h. After acidification with glacial acetic acid H$_2$O (20 mL) was added and the mixture was extracted with CH$_2$Cl$_2$ (2 × 20 mL). The organic layers were dried over sodium sulfate and evaporated under reduced pressure. The resulting yellow powder was used without further purification. ^{1}H NMR (CDCl$_3$): δ 4.0 (s, 2H, OCH_2COOH), 5.21 (s, 2H, C3′OCH_2Ph), 5.23 (s, 2H, C4′OCH_2Ph), 7.00 (d, 1H, *J* = 9 Hz, C5′H), 7.18−7.74 (m, 15H, 2× OCH$_2$*Ph* + C2′H + C6′H + C6H + C7H + C8H), 8.2 (dd, 1H, *J* = 8.0 Hz, 1 Hz, C5H).

3′,4′-Dibenzyloxy-3-tetraacetylglucosylflavone (6i). To

a solution of 1 mmol of **4b** and 1.5 mmol of acetobromoglucose in dry pyridine (10 mL) (+ molecular sieves 5 Å) was added 2.2 mmol of silver oxide. After stirring at room temperature for 1 h the silver salts were removed by filtration over Celite and silica. The solvent was removed in vacuo and the crude product was purified by column chromatography (ether) to give 700 mg (90%) of a green glassy solid. ^{1}H NMR (CDCl$_3$): δ 1.78 (s, 3H, Ac), 1.98 (s, 3H, Ac), 1.99 (s, 3H, Ac), 2.07 (s, 3H, Ac), 3.58 (dt, 1H, J = 10 Hz, 1 Hz, CH−O), 3.9−4.1 (m, 2H, CH$_2$-OAC), 5.0−5.3 (m, 3H, 3× CH−O), 5.24 (s, 2H, OC*H$_2$*Ph), 5.28 (s, 2H, OC*H$_2$*Ph), 5.68 (d, 1H, J = 8 Hz, acetal-H), 6.99 (d, 1H, J = 9 Hz, C5′H), 7.3−7.8 (m, 15H, 2× OCH$_2$*Ph* + C2′H + C6′H + C6H + C7H + C8H), 8.18 (dd, 1H, J = 8 Hz, 2 Hz, C5H).

3′,4′-Dibenzyloxy-3-hexaacetylrutinosylflavone (6j). A suspension of 5 g of rutine in 50% acetic acid (150 mL) was heated to reflux under an atmosphere of dry nitrogen for 6 h. H$_2$O (100 mL) was added and the reaction mixture was kept at 4 °C overnight and filtered. The filtrate was concentrated in vacuo. The residue was dissolved in pyridine (15 mL) and acetic acid anhydride (15 mL) and stirred for 3 h at room temperature. CH$_2$Cl$_2$ was added and the organic layer was washed with H$_2$O (2×), dried over sodium sulfate and evaporated to dryness. The product was purified by column chromatography (ether) to give 1.28 g (27%) of β-heptaacetylruti-nose as a white 'glass'.

This material was dissolved in dry CH$_2$Cl$_2$ (12 mL), 4.9 mmol of TiBr$_4$ in dry CH$_2$Cl$_2$ (12 mL) was added and the reaction mixture was stirred for 17 h at room temperature under an atmosphere of dry nitrogen. The reaction mixture was neutralized with NaOAc and filtered over Celite and concentrated under reduced pressure. The resulting orange/brown oil (1.6 g) was used without further purification. **4b** was reacted with α-acetobromorutinose as described for **6i** to give 634 mg (46%) **6j** as a white-greenish 'glass'. ^{1}H NMR (CDCl$_3$): δ 0.97 (d, 3H, J = 6 Hz, CH$_3$), 1.86 (s, 3H, Ac), 1.88 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.97 (s, 3H, Ac), 2.00 (s, 3H, Ac), 2.03 (s, 3H, Ac), 3.3−3.7 (m, 4H, 2× CH−O + CH$_2$O), 4.49 (s, 1H, acetal-H), 4.8−5.3 (m, 6H, CH−O), 5.21 (s, 2H, OC*H$_2$*Ph), 5.23 (s, 2H, OC*H$_2$*Ph), 5.65 (d, 1H, J = 8 Hz, acetal-H), 7.03 (d, 1H, J = 9 Hz, C5′H), 7.2−7.7 (m, 15H, 2× OCH$_2$*Ph* + C2′H + C6′H + C6H + C7H + C8H), 8.15 (d, 1H, J = 8 Hz, C5H).

General Procedure for Debenzylation (Method A). A suspension of 2 mmol of the protected product in HCl (36%, 50 mL) and glacial acid (50 mL) was heated to 100 °C under an atmosphere of dry nitrogen for 2−3 h. The solvents were evaporated under reduced pressure and the product was recrystallized.

3′,4′,7-Trihydroxyflavone (8a). Crystallized from ethanol; yield 82%; mp dec at 300 °C. ^{1}H NMR (CDCl$_3$ + 20% DMSO): δ 6.35 (br s, OH + H$_2$O), 6.59 (s, 1H, C3H), 6.8−6.9 (m, 3H, C8H, C6H, C5′H), 7.26 (d, 1H, J = 9 Hz, C6′H), 7.34 (s, 1H, C2′H), 7.86 (d, 1H, J = 8 Hz, C5H). Anal. (C$_{15}$H$_{10}$O$_5$) C, H.

3′,4′-Dihydroxy-7-methoxyflavone (8b). Crystallized from ethanol/chloroform 4:1 to give 0.5 g (88%) of yellow powder; mp > 240 °C. ^{1}H NMR (CDCl$_3$ + 20% DMSO): δ 3.87 (s, 3H, OCH$_3$), 6.50 (s, 1H, C3H), 6.8−7.0 (m, 3H, C5′H + C6H + C8H), 7.2−7.4 (m, 2H, C6′H + C2′H), 7.93 (d, 1H, J = 9 Hz, C5H). Anal. (C$_{16}$H$_{12}$O$_5$) C, H.

3′,4′-Dihydroxy-7-hydroxyethoxyflavone (8c). Yield 83%; mp 206.7−208.7 °C. ^{1}H NMR (DMSO): δ 3.76 (t, 2H, J = 5 Hz, CH$_2$OH), 4.13 (t, 2H, J = 5 Hz, C7OCH$_2$), 6.66 (s, 1H, C3H), 6.90 (d, 1H, J = 8 Hz, C5′H), 7.07 (d, 1H, J = 7 Hz, C6H), 7.28 (s, 1H, C8H), 7.35−7.50 (m, 2H, C2′H + C6′H), 7.89 (d, 1H, J = 8 Hz, C5H). Anal. (C$_{17}$H$_{14}$O$_6$) C, H.

N-(3-(3′,4′-Dihydroxyflavon-7-yl)oxypropyl)-N,N,N-trimethylammonium Chloride (8d). Crystallized from ethanol/diethyl ether yielding 0.8 g (91%) of **8d**; mp > 210 °C. ^{1}H NMR (DMSO + D$_2$O): δ 2.22 (m, 2H, (CH$_3$)$_3$NCH$_2$C*H$_2$*CH$_2$O), 3.12 (s, 9H, (C*H$_3$*)$_3$NCH$_2$CH$_2$CH$_2$O), 3.50 (m, 2H, (CH$_3$)$_3$NC*H$_2$*-CH$_2$CH$_2$O), 3.89 (m, 2H, (CH$_3$)$_3$NCH$_2$CH$_2$C*H$_2$*O), 6.26 (s, 1H, C3H), 6.55−7.2 (m, 5H, C5′H + C6′H + C8H + C5H + C6H), 7.58 (d, 1H, J = 9 Hz, C5H). Anal. (C$_{21}$H$_{24}$ClNO$_5$) C, H, N.

3′,4′-Dihydroxy-7-(3-dimethylaminopropoxy)flavone (8e). The residue was dissolved in CH$_2$Cl$_2$. The resulting

suspension was filtered and the residue gave 0.5 g (93%) of **8e**; mp 173.6 °C. ^{1}H NMR (D$_2$O): δ 2.02 (m, 2H, CH$_2$) 2.88 (s, 6H, N(CH$_3$)$_2$), 3.18 (t, 2H, J = 7 Hz, CH$_2$N), 3.60 (m, 2H, C7OCH$_2$), 5.70 (s, 1H, C3H), 6.10 (s, 1H, C8H), 6.30−6.60 (m, 4H, C6H + C2′H + C5′H + C6′H), 7.13 (d, 1H, J = 9 Hz, C5H). Anal. (C$_{20}$H$_{21}$NO$_5$·HCl) C, H, N.

3,3′,4′-Trihydroxyflavone (9a). Washed with hexane and ether (twice); yield 69%; mp dec at 280 °C. ^{1}H NMR (DMSO): δ 6.89 (d, 1H, J = 9 Hz, C5′H), 7.44 (t, 1H, J = 8 Hz, C6H), 7.61 (dd, 1H, J = 9 Hz, 2 Hz, C6′H), 7.65−7.75 (m, C7H + C8H + C2′H), 8.09 (d, 1H, J = 8 Hz, C5H), 9.4 (br s, 3H, OH). Anal. (C$_{15}$H$_{10}$O$_5$) C, H.

(3′,4′-Dihydroxyflavon-3-yl)oxyacetic Acid (9d). Yield 0.21 g (43%) of **9d** as a green powder; mp 253.3−254.9 °C. ^{1}H NMR (DMSO): δ 4.74 (s, 2H, OC*H$_2$*COOH), 6.89 (d, 1H, J = 9 Hz, C5′H), 7.48 (t, 1H, J = 7.4 Hz, C6H), 7.58−7.86 (m, 4H, C6′H + C7H + C8H + C2′H), 8.09 (dd, 1H, J = 8 Hz, 2 Hz, C5H), 9.32 (s, 1H, C3′OH), 9.79 (s, 1H, C4′OH), 12.92 (br s, 1H, CH$_2$COO*H*). Anal. (C$_{17}$H$_{12}$O$_7$) C, H.

N-(3-(3′,4′-Dihydroxyflavon-3-yl)oxypropyl)-N,N,N-trimethylammonium Mixed Bromide/Chloride Salt (9f). Crystallized from ethanol; yield 91%; mp 234.4−235.4 °C. ^{1}H NMR (D$_2$O): δ 1.86 (m, 2H, CH$_2$), 2.98 (s, 9H, N(CH$_3$)$_3$), 3.12 (m, 2H, CH$_2$N), 3.45 (t, 2H, J = 6 Hz, OCH$_2$), 6.49 (d, 1H, J = 9 Hz, C5′H), 6.74 (d, 1H, J = 8 Hz, C8H), 6.8−6.9 (m, 2H, C2′H + C6′H), 7.10 (t, 1H, J = 8 Hz, C6H), 7.37 (t, 1H, J = 8 Hz, C7H), 7.46 (d, 1H, J = 8 Hz, C5H). Anal. (C$_{21}$H$_{24}$NO$_5$Cl$_{0.62}$-Br$_{0.38}$) C, H, N.

N-(6-(3′,4′-Dihydroxyflavon-3-yl)oxyhexyl)-N,N,N-trimethylammonium Chloride (9g). Crystallized from ethanol; yield 51%; mp 230.8−232.8 °C. ^{1}H NMR (DMSO): δ 1.15−1.8 (m, 8H, CH$_2$), 3.04 (s, 9H, N$^+$(CH$_3$)$_3$), 3.36 (m, 2H, CH$_2$N), 3.98 (t, 2H, J = 6 Hz, OCH$_2$), 6.96 (d, 1H, J = 8 Hz, C5′H), 7.4−7.9 (m, 5H, C8H + C2′H + C6′H + C6H + C7H), 8.08 (dd, 1H, J = 8 Hz, 1 Hz, C5H), 9.44 (s, 1H, OH), 9.91 (s, 1H, OH). Anal. (C$_{24}$H$_{30}$ClNO$_5$) C, H, N.

N-(8-(3′,4′-Dihydroxyflavon-3yl)oxyoctyl)-N,N,N-trimethylammonium Bromide (9h). Debenzylation was carried out with HBr instead of HCl; washed with ethyl acetate; yield 54%; mp 196.7−198.5 °C. ^{1}H NMR (DMSO): δ 1.15−1.75 (m, 12H, CH$_2$), 3.04 (s, 9H, N$^+$(CH$_3$)$_3$), 3.28 (m, 2H, CH$_2$N), 3.97 (t, 2H, J = 6 Hz, OCH$_2$), 6.91 (d, 1H, J = 8 Hz, C5′H), 7.4−7.9 (m, 5H, C8H + C2′H + C6′H + C6H + C7H), 8.07 (dd, 1H, J = 8 Hz, 1 Hz, C5H), 9.44 (br s, 1H, OH), 9.67 (br s, 1H, OH). Anal. (C$_{26}$H$_{34}$BrNO$_5$) C, H, N.

3-Methoxy-7,3′,4′-trihydroxyflavone (10b). Crystallized from ethanol/chloroform (4:1) to give 0.5 g (90%) of a yellow powder; mp 281.4 °C. ^{1}H NMR (DMSO): δ 3.76 (s, 3H, OCH$_3$), 6.8−7.0 (m, 3H, C5′H + C6H + C8H), 7.42 (dd, 1H, J = 8 Hz, 1 Hz, C6′H), 7.55 (d, 1H, J = 1 Hz, C2′H), 7.86 (d, 1H, J = 8 Hz, C5H), 9.90 (br s, 1H, C7OH). Anal. (C$_{16}$H$_{12}$O$_6$) C, H.

3-Hydroxyethoxy-7,3′,4′-trihydroxyflavone (10c). Washed with CHCl$_3$ to give 0.5 g (80%) of an orange powder; mp >300 °C. ^{1}H NMR (DMSO): δ 4.66 (m, 2H, OCH$_2$C*H$_2$*OH), 5.00 (m, 2H, OC*H$_2$*CH$_2$OH), 7.05 (d, 1H, J = 8 Hz, C5′H), 7.35 (d, 1H, J = 8 Hz, C6H), 7.46 (s, 1H, C8H), 7.88−8.00 (m, 2H, C6′H + C2′H), 8.06 (d, 1H, J = 9 Hz, C5H), 9.88 (br s, 1H, C7OH), 10.85 (br s, 1H, C3′OH), 12.35 (br s, 1H, C4′OH). Anal. (C$_{17}$H$_{14}$O$_7$) C, H.

N-(3-(7,3′,4′-Trihydroxyflavon-3-yl)oxypropyl)-N,N,N-trimethylammonium Chloride (10d). Crystallized from methanol/ether (1:1) to give 95 mg (95%) of a brown-white powder; mp dec >200 °C. ^{1}H NMR (DMSO): δ 2.11 (m, 2H, CH$_2$), 3.07 (s, 9H, N$^+$(CH$_3$)$_3$), 3.48 (m, 2H, CH$_2$N), 3.97 (t, 2H, J = 6 Hz, OCH$_2$), 6.9−7.0 (m, 3H, C5′H + C6H + C8H), 7.43 (dd, 1H, J = 8 Hz, 2 Hz, C6′H), 7.54 (s, 1H, J = 2 Hz, C2′H), 7.88 (d, 1H, J = 9 Hz, C5H), 9.56 (s, 1H, OH), 9.76 (s, 1H, OH), 10.93 (s, 1H, OH). Anal. (C$_{21}$H$_{24}$ClNO$_6$) C, H, N.

General Procedure for Debenzylation (Method B). To a suspension/solution of 1 mmol of the required compound in methanol (100 mL) or a mixture of ethyl acetate/methanol (2:1) was added 150 mg of 10% Pd/C. The reaction mixture was stirred at room temperature for 30 min to 1 h under an atmosphere of 1.5 atm H$_2$(g) after replacement of the air by

nitrogen. The Pd/C was filtered and the solvent was evaporated under reduced pressure.

3′,4′-Dihydroxy-3-methoxyflavone (9b). Yield 88%; mp 198.5−199.7 °C. ^{1}H NMR (DMSO): δ 3.83 (s, 3H, OCH$_3$), 6.95 (d, 1H, J = 9 Hz, C5′H), 7.45−7.88 (m, 5H, C7H + C6H + C8H + C6′H + C2′H), 8.1 (d, 1H, J = 7 Hz, C5H). Anal. (C$_{16}$H$_{12}$O$_5$) C, H.

3′,4′-Dihydroxy-3-(hydroxyethoxy)flavone (9c). Yield 85%; mp 186.2−186.8 °C. ^{1}H NMR (DMSO): δ 3.65 (t, 2H, OCH$_2$CH_2OH), 4.05 (t, 2H, OCH_2CH$_2$OH), 6.9 (d, 1H, J = 9 Hz, C5′H), 7.45 (dt, 1H, J = 7 Hz, 1 Hz, C6H), 7.6−7.85 (m, 4H, C7H + C8H + C6′H + C2′H) 8.08 (dd, 1H, J = 7 Hz, 1 Hz, C5H). Anal. (C$_{17}$H$_{14}$O$_6$) C, H.

3′,4′-Dihydroxy-3-(3-dimethylaminopropoxy)flavone Hydrochloride (9e). Crystallized from methanol and washed with ether; yield 150 mg (21% over 2 steps); mp 98.5−99.8 °C. ^{1}H NMR (CDCl$_3$ + 20% DMSO): δ 2.11 (t, 2H, OCH$_2$CH_2-CH$_2$N(CH$_3$)$_2$), 2.74 (s, 6H, N(CH$_3$)$_2$), 3.24 (m, 2H, CH$_2$N), 3.85 (t, 2H, J = 6 Hz, OCH_2CH$_2$CH$_2$N(CH$_3$)$_2$), 6.79 (d, 1H, J = 8 Hz, C5′H), 7.2−7.6 (m, 4H, C6H + C8H + C6′H + C7H), 7.70 (d, 1H, J = 2 Hz, C2′H), 7.95 (dd, 1H, J = 8 Hz, 1 Hz, C5H), 8.56 (s, 1H, OH), 8.89 (s, 1H, OH), 11.35 (br s, 1H, N$^+$H). Anal. (C$_{20}$H$_{21}$NO$_5$·HCl) C, H, N.

3′,4′-Dihydroxy-3-glucosylflavone (9i). Before the debenzylation procedure, the acetyl groups were removed. 0.6 mmol of potassium *tert*-butoxide was added to a solution of 380 mg (0.49 mmol) of **6i** in MeOH (50 mL) and the reaction mixture was stirred for 1.5−2 h. H$_2$O was added and the water layer was extracted twice with ethyl acetate. The crude product was purified by column chromatography (ethyl acetate/methanol 9:1) to give 280 mg (94%). Subsequently the compound was debenzylated.; yield 89%; mp 166.6−168.6 °C. ^{1}H NMR (DMSO): δ 3.0−3.7 (m, 6H, 4× CH + 1× CH$_2$ sugar), 5.55 (m, 1H, acetal-H), 6.86 (d, 1H, J = 9 Hz, C5′H), 7.48 (t, 1H, J = 7 Hz, C6H), 7.6−7.9 (m, 4H, C2′H + C6′H + C7H + C8H), 8.08 (d, 1H, J = 8 Hz, C5H). ^{13}C NMR (DMSO): 60.7 (CH$_2$), 69.6 (CH), 73.7 (CH), 76.3 (CH), 77.3 (CH), 100.5 (acetal-CH), 114.9 (CH), 116.2 (CH), 117.9 (CH), 121.2 (C), 121.4 (CH), 123.0 (C), 124.7 (2× CH), 133.6 (CH), 135.4 (C), 144.6 (C), 148.1 (C), 154.2 (C), 155.7 (C), 173.1 (CO). Anal. (C$_{21}$H$_{20}$O$_{10}$) C, H.

3′,4′-Dihydroxy-3-rutinosylflavone (9j). Before the debenzylation procedure the acetyl groups were removed as described for **9i**; yield 95%; mp dec at 174 °C. ^{1}H NMR (DMSO): δ 0.97 (d, 3H, J = 6 Hz, CH$_3$) 3.0−3.7 (m, 10H, 8× CH + 1× CH$_2$ sugar), 5.46 (m, 2H, 2× acetal-H), 6.86 (d, 1H, J = 9 Hz, C5′H), 7.46 (t, 1H, J = 7 Hz, C6H), 7.6−7.9 (m, 4H, C2′H + C6′H + C7H + C8H), 8.08 (d, 1H, J = 8 Hz, C5H). ^{13}C NMR (DMSO): δ 17.5 (CH$_3$), 66.6 (CH$_2$), 68.0 (CH), 69.8 (CH), 70.1 (CH), 70.2 (CH), 71.6 (CH), 73.7 (CH), 75.7 (CH), 76.4 (CH), 100.4 (acetal-CH), 100.8 (acetal-CH), 115.0 (CH), 116.2 (CH), 117.9 (CH), 121.2 (C), 121.5 (CH), 122.9 (C), 124.7 (2× CH), 133.6 (CH), 135.4 (C), 144.5 (C), 148.0 (C), 154.3 (C), 156.0 (C), 173.2 (CO). Anal. (C$_{27}$H$_{30}$O$_{14}$) C, H.

7-(2-Hydroxyoxyethoxy)-3,3′,4′-trihydroxyflavone (11a). Yield 240 mg (92%); mp dec at 300 °C. ^{1}H NMR (DMSO): δ 3.77 (q, 2H, J = 6 Hz, HOCH_2CH$_2$O), 4.15 (t, 2H, J = 6 Hz, HOCH$_2$CH_2O), 4.99 (t, 1H, J = 6 Hz, HOCH$_2$CH$_2$O), 6.89 (d, 1H, J = 9, C5′H), 7.05 (dd, 1H, J = 9 Hz, 2 Hz, C6H), 7.22 (d, 1H, J = 2 Hz, C8H), 7.55 (dd, 1H, J = 8 Hz, 2 Hz, C6′H), 7.74 (d, 1H, J = 2 Hz, C2′H), 7.97 (d, 1H, J = 9 Hz, C5H), 9.17 (1H, s, C3OH), 9.27 (s, 1H, C3′OH), 9.57 (s, 1H, C4′OH). Anal. (C$_{17}$H$_{14}$O$_7$) C, H.

Preparation of Microsomes. Male Wistar rats (200−220 g) obtained from Harlan Olac CPB (Horst, The Netherlands) were sacrificed by decapitation. Livers were removed and homogenized in ice-cold phosphate buffer (50 mM phosphate + 0.1 mM EDTA, pH = 7.4; 1:2 w/v). The homogenate was centrifuged at 10000g (20 min at 4 °C). Subsequently the supernatant was centrifuged at 100000g for 60 min (4 °C). The pellet was resuspended in phosphate buffer and centrifuged again at 100000g (60 min, 4 °C). Finally the microsomal pellet was resuspended in phosphate buffer (microsomes originating from 2 g liver/mL buffer) and 1-mL aliquots were stored at −80 °C.

LPO Assay. Microsomes were thawed and resuspended in ice-cold Tris-HCl buffer (50 mM, pH = 7.4 at 37 °C) and washed by centrifugation (45 min, 115000g). The pellet was resuspended in 2 mL of 50 mM Tris-HCl buffer and heated to 100 °C for at least 2 min to remove all enzymatic factors just before resuspending and diluting it in ice-cold Tris buffer to 1.5 mg/mL.

Stock solutions of the flavonoids were freshly prepared in nitrogen-purged DMSO and Nanopure water (1:1) just before use, as some of the flavonoids tended to oxidize very quickly. All compounds were added on ice, after which the incubates were transferred to a water bath (37 °C), where 200 μM ascorbate was added. The reaction was started by adding 10 μM freshly prepared FeSO$_4$ in nitrogen-purged Nanopure water. The maximum DMSO concentration in the final incubation mixture was 2.5%, which was found to have no influence on the assay. LPO was assayed by measuring thiobarbituric acid (TBA) reactive substances according to Haenen et al.[38] At t = 0, 5, 10, 15, 30, 45 and 60 min a 0.3-mL aliquot of the incubation was mixed with 2 mL of an ice-cold TBA−trichloroacetic acid−HCl−butylhydroxytoluene (BHT) solution to stop the reaction. After heating (15 min, 80 °C) and centrifugation (15 min) the absorbance at 535−600 nm was determined. The TBA−trichloroacetic acid−HCl−BHT solution was prepared by dissolving 41.6 mg of TBA/10 mL of trichloroacetic acid (16.8% w/v in 0.125 N HCl). To 10 mL of TBA−trichloroacetic acid−HCl was added 1 mL of BHT (1.5 mg/mL ethanol).

The IC$_{50}$ was determined by measuring the % LPO inhibition at several concentrations and interpolating the 50% inhibition point. The results are expressed as mean ± SEM (n = 3−5).

TEAC Assay. A solution of 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS; Sigma, St. Louis, MO) (1.23 mg/mL) and azobis(amidinepropane) (ABAP; Polysciences Inc., Warrington, MA) (3.96 mg/mL) in 50 mM phosphate buffer (pH = 7.4) was heated at 70 °C for 20 min. The amount of ABTS radicals was measured by determination of the absorbance at 734 nm.

Stock solutions of the flavonoids were freshly prepared in nitrogen-purged DMSO and Nanopure water (1:1) just before use. A callibration curve of trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Aldrich, Milwaukee, WI) was made in a concentration range from 0 to 20 μM. The maximum DMSO concentration in the final incubation mixture was 0.25%, which was found to have no influence on the assay. A mixture of 50 μL of test compound and 950 μL of ABTS$^{•+}$ solution was incubated at 37 °C for 5 min. Subsequently the absorbance at 734 nm was determined on a UV/VIS spectrophotometer (Ultrospec 2000, Amersham Pharmacia Biotech, Sweden).

The antioxidant-induced decrease in absorbance is directly related to the antioxidant capacity of the compound (solution) being tested. The trolox equivalent antioxidant capacity (TEAC) is defined as the concentration (mM) of trolox having an antioxidant capacity equivalent to 1 mM test compound. The data are expressed as mean ± SD (n = 3−5).

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