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Original article

Linear and branched alkyl-esters and amides of gallic acid and other (mono-, di- and tri-) hydroxy benzoyl derivatives as promising anti-HCV inhibitors

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ABSTRACT

Linear and branched compounds that contain two, three or five units of galloyl (3,4,5-trihydroxybenzoyl) or its isomer 2,3,4-trihydroxybenzoyl, as well as other mono- or dihydroxybenzoyl moieties have been synthesized. These molecules have been evaluated for their *in vitro* inhibitory effects against a wide panel of viruses showing preferential activity against HIV and HCV.

Our structure-activity relationship studies demonstrated that the 2,3,4-trihydroxybenzoyl moiety provides better antiviral activities than the galloyl (3,4,5-trihydroxybenzoyl) moiety that is present in natural green tea catechins. This observation can be of interest for the further rational exploration of compounds with anti-HCV/HIV properties.

The most notable finding with respect to HIV is that the tripodal compounds **43** and **45**, with three 2,3,4-trihydroxybenzoyl moieties, showed higher activities than linear compounds with only one or two. With respect to HCV, the linear compounds, **52** and **41**, containing a 12 polymethylene chain and two 2,3 di- or 2,3,4 tri-hydroxybenzoyl groups respectively at the ends of the molecule showed good antiviral efficiency. Furthermore, the anti-HCV activity of both compounds was observed at concentrations well below the cytotoxicity threshold. A representative member of these compounds, **41**, showed that the anti-HCV activity was largely independent of the genetic make-up of the HCV subgenomic replicon and cell lines used.

1. Introduction

Several chronic viral infections (such as AIDS and hepatitis C) are highly prevalent and a serious health risk for which there is no curative treatment and no vaccine.

Hepatitis C virus (HCV) belongs to the family of *Flaviviridae* and is an important aetiological agent causing chronic hepatitis that can progress further to hepatocellular carcinoma [1]. A "dual therapy" based in the combination of pegylated interferon-alpha (PEG-IFNα) and ribavirin was the standard therapy of chronic HCV infection for more than 10 years [2]. In recent years the advances in the knowledge of the hepatitis C life cycle resulted in the development of different classes of direct-acting antiviral (DAA) agents [3]. These drugs act as inhibitors of different non-structural HCV proteins such as: NS3/4A protease, NS5BRNA-dependent RNA polymerase and NS5A. In 2011, the first two DDAAs, telaprevir and boceprevir, targeting the NS3/4A protease of HCV, have been approved by the FDA for chronic HCV infection [4]. These drugs, used in combination with peginterferon and ribavirin as a "triple therapy", have significantly increased the sustained viral response rate for patients infected with genotype 1 viruses (~75% *versus* ~ 45%, in the "dual" combination therapy). Despite this major therapeutic success the "triple therapy" is associated with numerous side effects and limited to genotype 1 viruses [5]. These limitations highlighted the

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urgent need for new lead compounds and novel therapeutic concepts to fight against Hepatitis C infection.

With respect to infection with Human Immunodeficiency virus (HIV), the introduction of highly active antiretroviral therapy (HAART), in the late 1990s, have significantly contribute to reduce the morbidity and mortality due to infection with this virus. However, issues such as long-term toxicities, drug–drug interactions and emergence and transmission of drug-resistant viral strains represent a serious problem to long-term successful treatment that justify the search for new therapeutic approaches and drugs for the treatment of this infection [6].

Polyphenols or vegetable tannins are a diverse group of natural compounds characterized by the presence of multiple phenolic functionalities per molecule [7]. They are secondary metabolites produced by higher plants as a defense strategy against invading pathogens including insects, bacteria, fungi and viruses or as a response to environmental stress [8]. In addition, polyphenols serve to dissuade herbivores from eating plants due to their unpleasant and anti-nutritional properties [9].

Green tea (*Camellia sinensis*), one of the most popular beverages in the world, is an important source of polyphenols, especially flavonoids of the catechin-type. Among them, (−) epigallocatechin gallate (EGCG) is the most abundant (40–60%), followed by (−) epicatechin gallate (ECG, 10–20%), (−) epigallocatechin (EGC, 10–20%) and (−) epicatechin (EC, 4–6%) [10].

The green tea catechins are well-known for their antioxidant properties [11] and their beneficial therapeutic effects against some forms of cancer [12] cardiovascular [13] and neurological diseases [14]. In addition many antimicrobial activities [15], such as antibacterial [16], antifungal [17] and antiviral [18] have been described for this group of molecules.

Nowadays it is generally accepted that the pyrogallol and galloyl groups, present in several natural tea catechins, are responsible for most of their antioxidant, anticancer and antimicrobial activities [19,20]. In fact, EGCG and ECG, having a 3-galloyl side-chain, showed higher antimicrobial activity and anticancer properties than catechins, like EC and EGC, that lack the galloyl moiety.

In the light of the wide range of biological activities attributed to natural green tea catechins they have been used as lead structures for the development of synthetic analogs. Among them, n-alkyl esters of gallic acid, also known as alkyl gallates, have become a focus of attention because of their antioxidant properties. In fact, they have been widely employed as antioxidant additives in both, food and pharmaceutical industry [21]. In addition, there are numerous studies demonstrating that alkyl gallates have a wide range of biological activities including anticancer [22], antibacterial [23], antifungal [24] and antiviral activity [25].

Taking into account that the pyrogallol and galloyl groups present in natural tea catechins and synthetic alkyl gallates are responsible for most of their biological activities we considered of particular interest the combination of two or more of these galloyl moieties, or other related phenolic substituents, in a single molecule as a novel class of synthetic alkyl gallates. We reasoned that this approach would allow obtaining molecules with enhanced biological activity with respect to the "classical" alkyl gallates (with only one galloyl residue per molecule). Here we report the synthesis of binary, tri- and penta-alkyl gallate analogs and their antiviral effects against a wide variety of viruses.

In these compounds gallic acid (3,4,5-trihydroxybenzoic acid) and its isomer 2,3,4-trihydroxybenzoic acid, together with other mono- and dihydroxybenzoyl residues have been used as phenolic substituents while linear or branched polyamines or polyalcohols of different lengths and architectures were used as scaffolds. These scaffolds allowed the introduction, on the same molecule of a variable number of phenolic residues (from two to five) and the modification of the distance and spatial distribution between them (Fig. 1).

To determine the impact of the chemical structure on the antiviral activity we studied: i) the nature of the linkage between the gallic acid residues and the hydrocarbon chain (ester or amide); ii) the number of galloyl units bound to a given scaffold; iii) the number and position of OH groups on the aromatic ring.

2. Results and discussion

2.1. Chemical results

Firstly, several members of the well-known n-alkyl esters of gallic acid (alkyl gallates) (**2**–**7**) have been synthesized in our laboratory (Scheme 1) in order to be used as reference compounds in the biological evaluation. In our hands microwave conditions [26] prove superior than other more classical conditions [25d,27]. Briefly, gallic acid **1** with the appropriate alcohol and trace amounts of concentrated sulfuric acid as catalyst were microwave-irradiated during 50 min at 100 °C to give gallates **2**–**7** with excellent yields (82%–98%). Compared with the classical synthetic route, microwave conditions significantly reduced the reaction time (50 min at 100 °C *versus* 2–12 h at 160 °C), increase the yields (58%–80% *versus* 82%–98%) and simplify the experimental protocol (use of Dean Stark or vacuum).

With the aim to evaluate the relationship between the number of polyphenol moieties per molecule and the antiviral properties, a variable number (2, 3 and 5) of polyphenol residues were attached to different central scaffolds.

First, we prepared compounds **8**–**11** with two gallic acid residues at the terminal ends of the molecule (Scheme 2). To evaluate the influence of the length of the alkyl chain on the antiviral activity, different chain lengths were used to connect the two polyphenol subunits. It should be mentioned that the synthesis of some of these compounds has been previously described [22a,22b,28], however their antiviral activities were completely unknown. In the reported synthetic procedure a six-step route, involving protection of the phenolic functionalities, has been used. In our case a direct and shorter synthetic route involving only one-step reaction and non-protected phenol functionalities was attempted. Thus, in this route two equivalents of gallic acid was treated with the corresponding diols (1,4-butanodiol, 1,6-hexanodiol, 1,8-octanodiol and 1,12-dodecanodiol) in the presence of a catalytic amount of *p*-toluene sulfonic acid. The mixture was heated under reflux (160 °C) resulting in compounds **8**–**11** with 28%–70% yields. Microwave-assisted conditions was also attempted for the synthesis of these compounds, however complex mixtures of compounds were obtained.

Next, alkyl gallamides **18**–**22**, containing amide bonds instead of ester bonds and various lengths of the alkyl chain, were also prepared. As described in Scheme 3, these compounds were synthesized by condensation of the commercially available OMe-protected gallic acid chloride **12** with the corresponding diamines (1,4-butanodiamine, 1,6-hexanodiamine, 1,8-octanodiamine, 1,10-decanodiamine and 1,12-dodecanodiamine). Reactions were performed in the presence of potassium carbonate using a biphasic system of ethyl acetate/water. This reaction allowed the synthesis of the OMe-protected derivatives **13**–**17** in excellent yields (77%–98%). Deprotection of the methyl ether derivatives using the Lewis acid boron tribromide gave the deprotected final compounds **18**–**22** in moderate to good yields (51–84%).

The same synthetic methodology was applied for the synthesis of the OMe protected trigalloyl (**23**, **25** and **27**) (Scheme 4) and pentagalloyl **29** (Scheme 5) derivatives that were obtained in 38%–85% yields. In this case, diethylenetriamine, spermidine, tris(2-

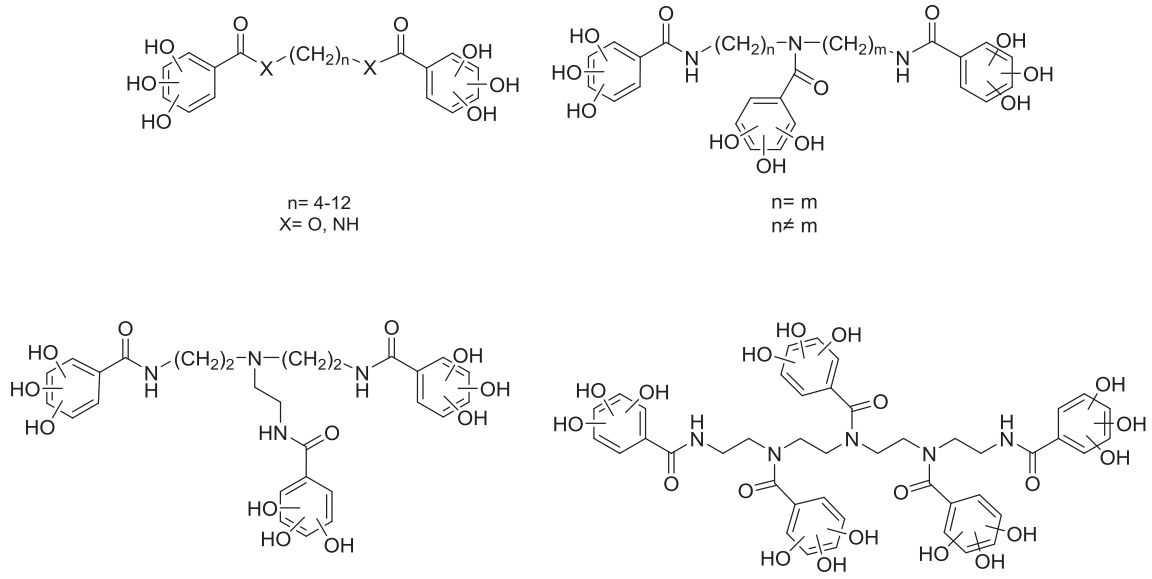
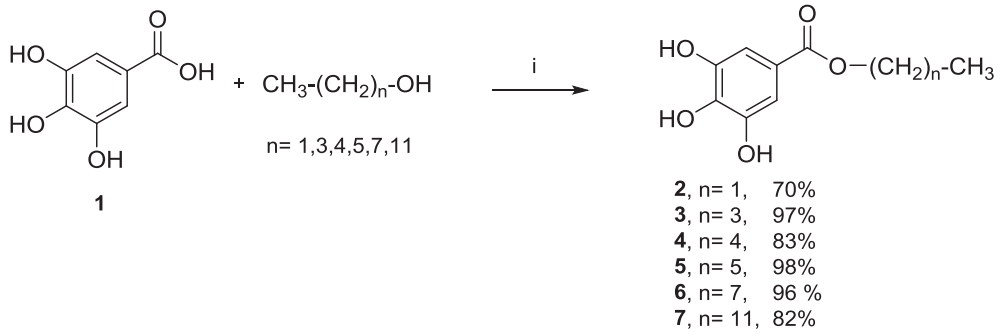
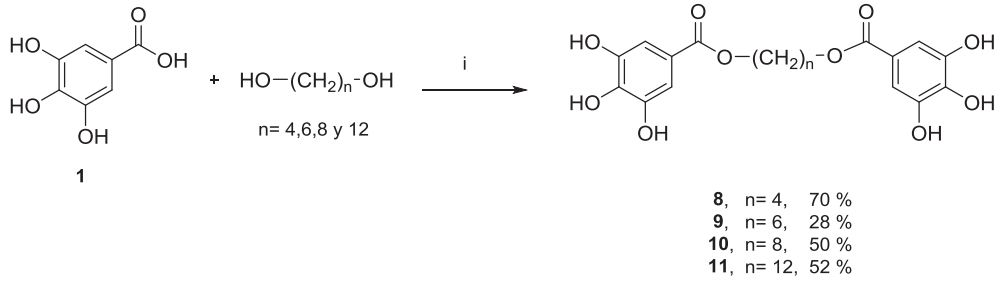


Fig. 1. Target linear and branched polyphenols.



Scheme 1. Synthesis of alkyl gallates **2**–**7** under microwave–irradiated conditions. *Reagents and conditions*: (i) MW, H_2SO_4 (cat.), 100 °C, 50 min.

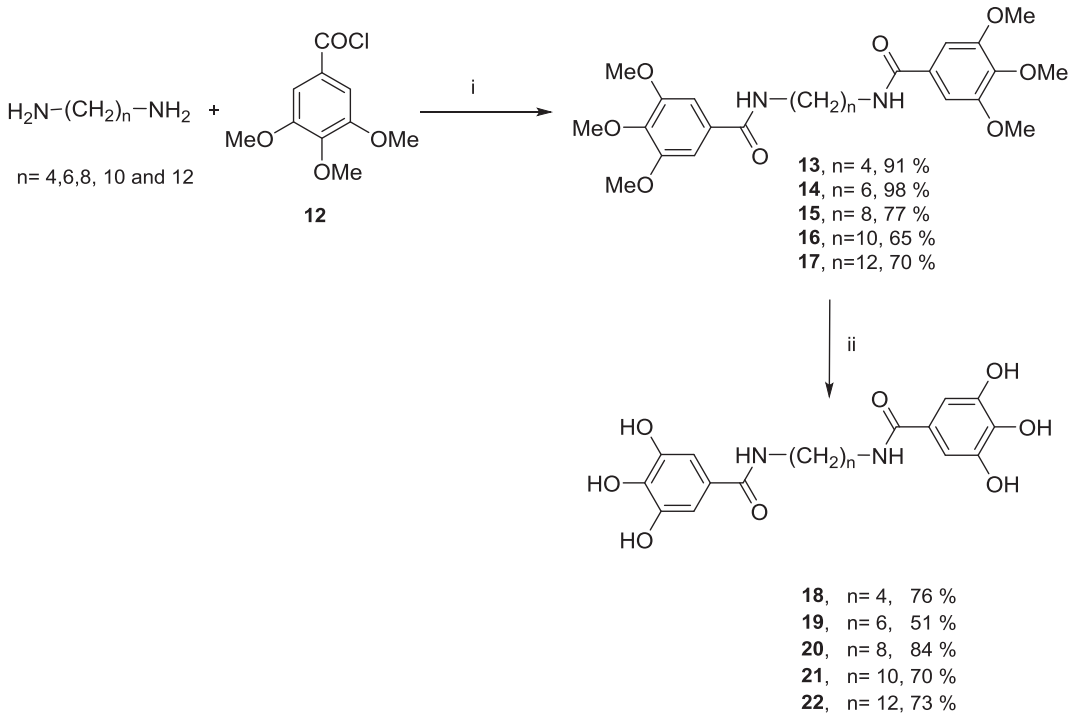


Scheme 2. Synthesis of alkyl digallates **8**–**11**. *Reagents and conditions*: (i) *p*-TSA (cat.), dioxane, 160 °C, 50 min.

aminomethyl)amine and tetraethylenepentamine were used as starting amines. Deprotection of the methyl ether derivatives **23**, **25**, **27** and **29** using the Lewis acid boron tribromide gave the corresponding deprotected final compounds **24**, **26**, **28** [29] and **30** in moderate to good yields (30–100%).

In order to investigate the effect of the spatial distribution of the hydroxyl groups on the aromatic ring, the corresponding 2,3,4-trihydroxybenzoyl isomeric derivatives **32**–**45** (Scheme 6) were also synthesized. In this case, the 2,3,4-benzyl protected intermediate **31** was employed as starting compound (Scheme 6). In turn, intermediate **31** was prepared starting from commercially available 2,3,4-trihydroxycarboxylic acid by perbenzylation of the carboxylic acid and hydroxyl groups followed by saponification of the ester group [30]. Next, condensation of **31** with the corresponding alkyl amine in the presence of pyBOP and triethylamine, followed by deprotection of the benzyl ether derivatives using H_2/Pd–C gave the deprotected final compounds **37**–**41**, **43** and **45** in moderate to good yields (35–61%). Synthesis of compound **45** was described in the literature [29c] using a 2-mercaptothiazoline 2,3,4-trimethoxy benzoic acid derivative, instead of the benzyl protected intermediate **31** used by us.

Next, to determine the impact of the number of hydroxyl groups present on the aromatic ring on the antiviral activity compounds **50**–**52** and **57**–**59**, with two or one hydroxyl groups, respectively



Scheme 3. Synthesis of alkyl digallamides **18**–**22**. *Reagents and conditions*: (i) K_2CO_3, AcOEt/H_2O (1:1) (ii) BBr_3, CH_2Cl_2.

were prepared (Schemes 7 and 8).

The dihydroxy-benzoyl analogs **50**–**52** (Scheme 7) were previously synthesized by different authors [31] although their antiviral properties were completely unknown. In our case a modification of the previously described procedures, that implies the use of pyBOP as coupling reagent, has been used for the synthesis of these compounds. In this reaction, the commercially available 2,3-dimethoxybenzoic acid **46** (Scheme 7) was used as starting material. Reaction of this intermediate with ethylene, tetramethylene and dodecamethylene diamines in the presence of pyBOP and triethylamine, followed by deprotection of the methyl ether derivatives **47**–**49** using the Lewis acid boron tribromide gave the deprotected final compounds **50**–**52** with excellent yields (81–99%).

The coupling reagent, pyBOP, was also used for the synthesis of the monohydroxy benzoyl analogs **57**–**59** (Scheme 8). In this case the commercially available 2-benzyloxy salicylic acid **53** was used as starting material. Reaction of this intermediate with the corresponding alkyl amine in the presence of pyBOP, as coupling reagent, and triethylamine followed by deprotection of the benzyl ether derivatives **54**–**56**, using H_2/Pd–C, gave the deprotected final compounds **57**–**59** with good to excellent yields (69–92%).

As it will be later discussed in the Antiviral Evaluation section, alkylgallamide **41** (Scheme 6), containing a twelve methylene linker connecting two phenolic subunits at the ends, showed significant anti-HCV activity in cell culture. To complete the structure-activity relationship studies of this compound we prepared compounds **61**, with a less lipophilic linker, and **63** with the phenolic OHs protected as OMe (Scheme 9). The synthesis of these compounds were accomplished by coupling of intermediates **31** and **62** with the corresponding amines in the presence of pyBOP ant triethylamine.

2.2. Biological results

Firstly, the new synthetic phenolic derivatives have been evaluated for their *in vitro* inhibitory effects against a wide panel of viruses such as: vaccinia, vesicular stomatitis, Coxsackie B4, respiratory syncytial, para-influenza-3, reovirus, Sindbis, influenza A (H1N1, H3N2), Punta Toro, feline corona, feline herpes and Chikungunya. None of these compounds showed activity against these viruses.

Next, the new synthetic compounds were evaluated against HIV. Gallic acid (**1**) and the "classical" alkyl gallates **2**–**7** were also included for comparative purpose. Dextran sulfate-5000 (DS-5000), pradimicin A and tenofovir acted as relevant controls. DS-5000 is a negatively charged HIV adsorption inhibitor, pradimicin A represents an uncharged gp120 carbohydrate-binding cyclic entry inhibitor and tenofovir is an HIV RT inhibitor.

None of the OMe- or OBn-protected compounds proved active at subtoxic concentrations (data not shown). These data point to the importance of unsubstituted phenolic OHs for activity.

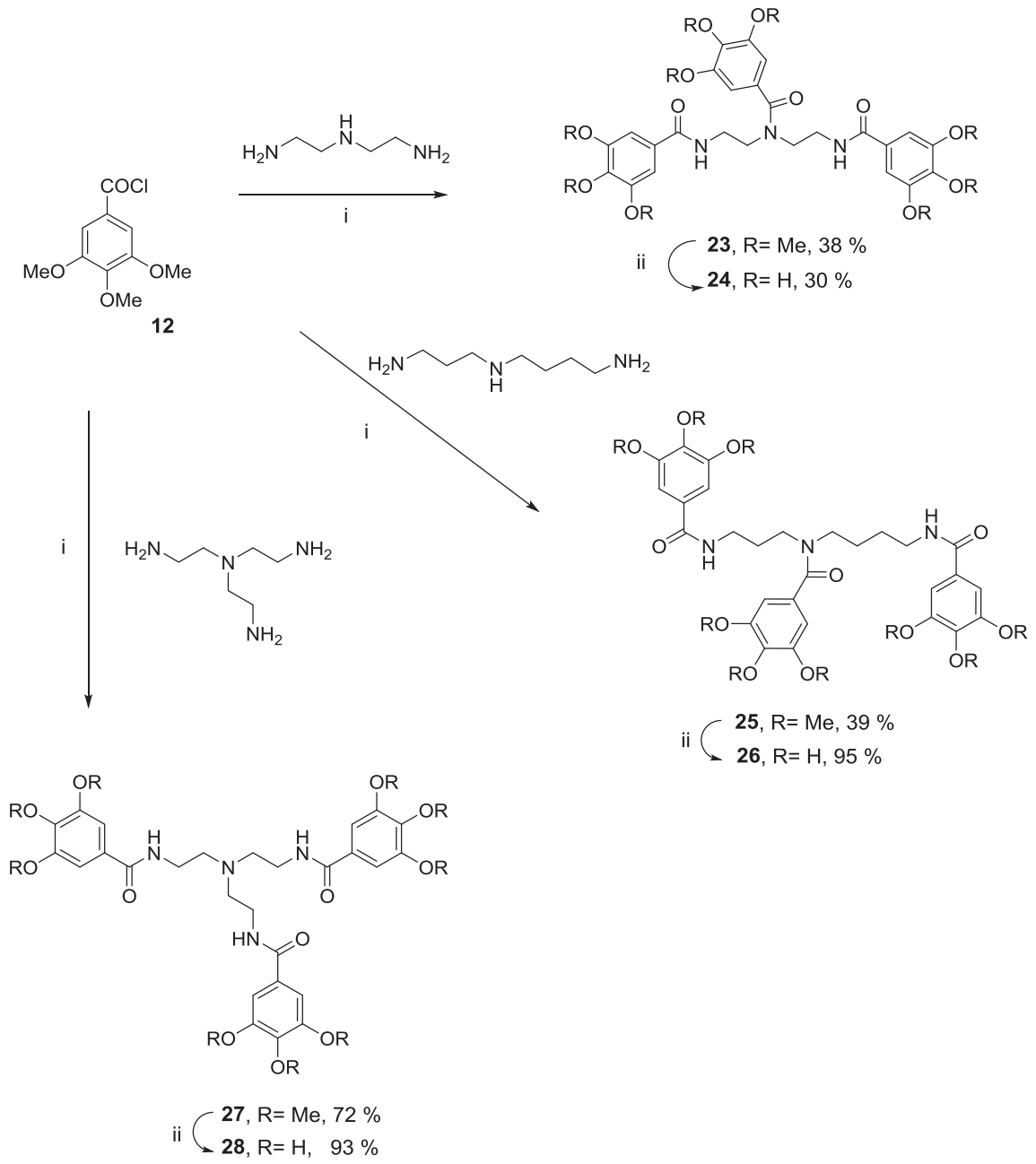
Table 1 summarizes the results of the anti-HIV evaluation of the deprotected compounds.

As it was shown in Table 1 gallic acid (**1**) did not show any anti-HIV activity up to 50 μM.

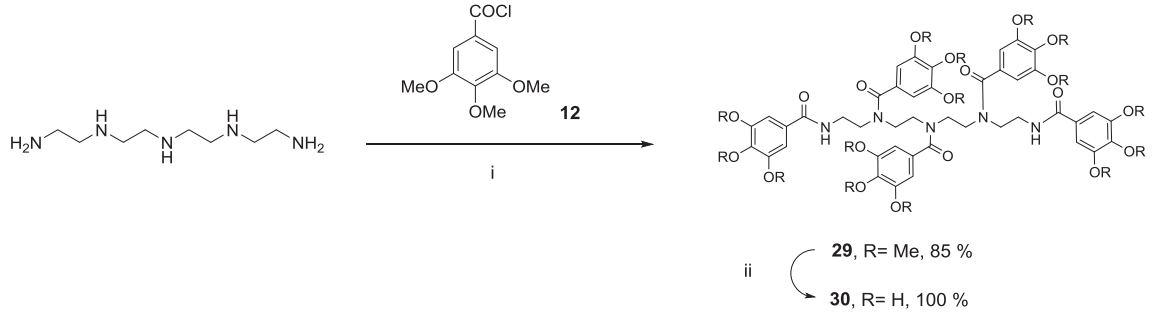
Within the group of alkyl gallates, compounds **2** to **7**, only pentyl and hexyl gallates (**4** and **5**) were endowed with anti-HIV-1 and -HIV-2 activity below, but close to subtoxic concentrations. Although their activity is close to the toxicity threshold (antiviral selectivity index ~ 2–4) these data are important because they represent a first hint of activity in this compound series. Moreover, and taking into consideration that gallic acid as such did not exhibit any antiviral activity against HIV, this result indicates that the alkyl moiety plays an important role in the eventual antiviral activity.

The digallates **8** to **11** could not improve the activities obtained for the monoesters.

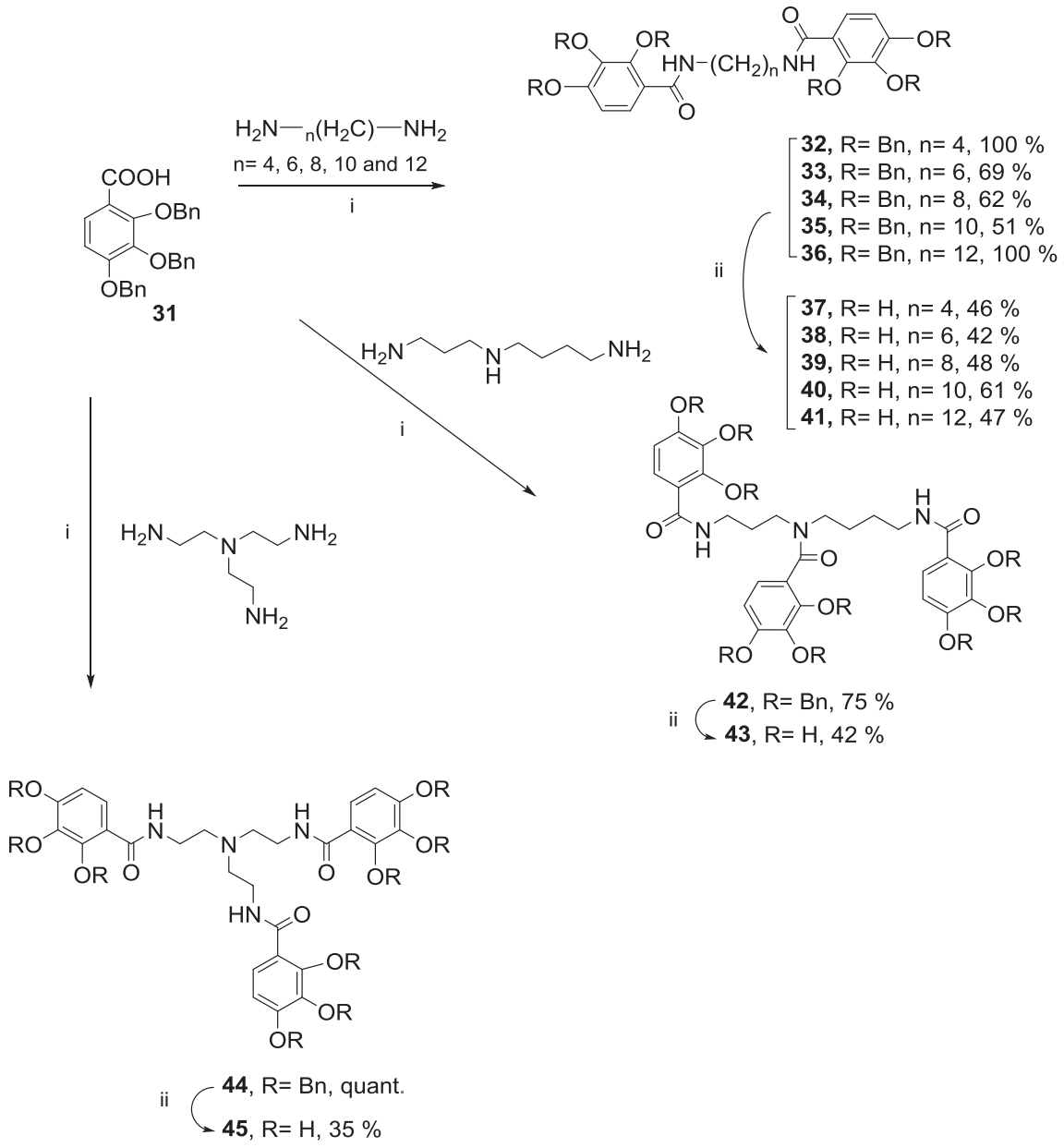
Within the group of alkyl digallamides (**18**–**22**), the most active compound was **18**, with a spacer of 4 methylenes, followed by **22** with a chain of 12 methylenes, as spacer. Again, the antiviral selectivity index was around 3 to 4, as observed for the alkyl gallates.



Scheme 4. Synthesis of branched trigallamides **24**, **26** and **28**. *Reagents and conditions*: (i) K_2CO_3, AcOEt/H_2O (1:1) (ii) BBr_3, CH_2Cl_2.



Scheme 5. Synthesis of pentagallamide **30**. *Reagents and conditions*: (i) K_2CO_3, AcOEt/H_2O (1:1) (ii) BBr_3, CH_2Cl_2.



Scheme 6. Synthesis of 2,3,4-trihydroxybenzoyl derivatives **32**–**45** *Reagents and conditions*: (i) pyBOP, Et_3N (ii) H_2, Pd/C.

Within the group of compounds with 3 (**24**, **26**, **28**) or 5 (**30**) units of gallic acid, the most active agent was **30** (EC_{50} HIV-1: 4.9 μM; selectivity index ~ 4). The spermidine derivative **26** showed an EC_{50} of 18 μM, but proved the least toxic compound in this series (CC_{50}: 107 μM), resulting in a selectivity index of ~6.

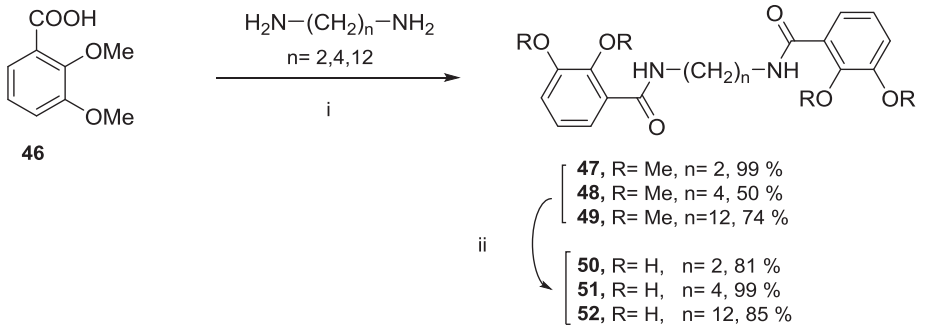
The alkyl amides **37** to **41**, with only two phenolic rings, were inactive at subtoxic concentrations. Interestingly, branched compounds **43** and **45**, bearing 3 phenolic rings with hydroxyl groups at positions 2,3,4, were endowed with the most pronounced anti-HIV activity among all compounds evaluated (EC_{50}: 2.5–2.6 μM). Activity showed by these compounds are below the toxicity threshold (CC_{50}: 27–28 μM)), resulting in selectivity indexes of ~10).

The compounds **50**–**52** and **57**–**59** in which gallic acid has been replaced by aromatic rings with only 2 or 1 hydroxyl groups, respectively, were inactive.

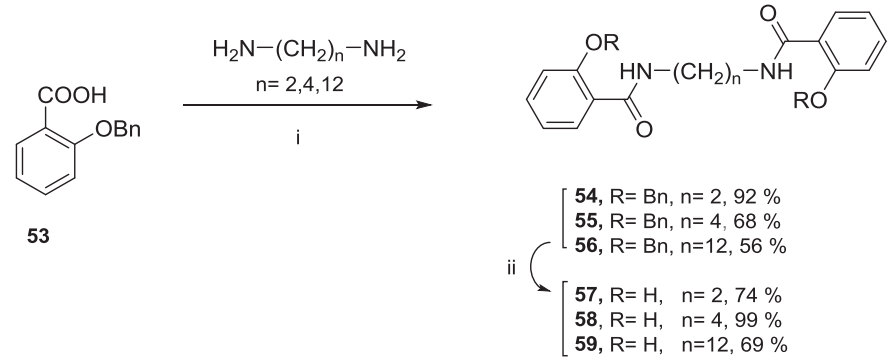
Importantly, the spermidine derivative **26**, substituted with 3 gallic acid moieties was less active than compound **43** substituted with three 2,3,4-trihydroxyphenyl rings. Similarly, compound **28** containing a tris(2-aminoethyl)amine skeleton substituted with 3 gallic acid moieties proved less active than **45** with the same skeleton and three 2,3,4-trihydroxy phenyl rings showing that the 2,3,4-trihydroxyphenyl moiety provides better anti-HIV activities than the gallic acid moiety.

Next, the new synthetic polyphenols were also evaluated for their *in vitro* inhibitory effects on hepatitis C virus replication. Table 2 summarizes the results of the anti-hepatitis C virus evaluation of the test compounds. The HCV NS3 protease inhibitor (Telaprevir, VX-950) was included as positive control.

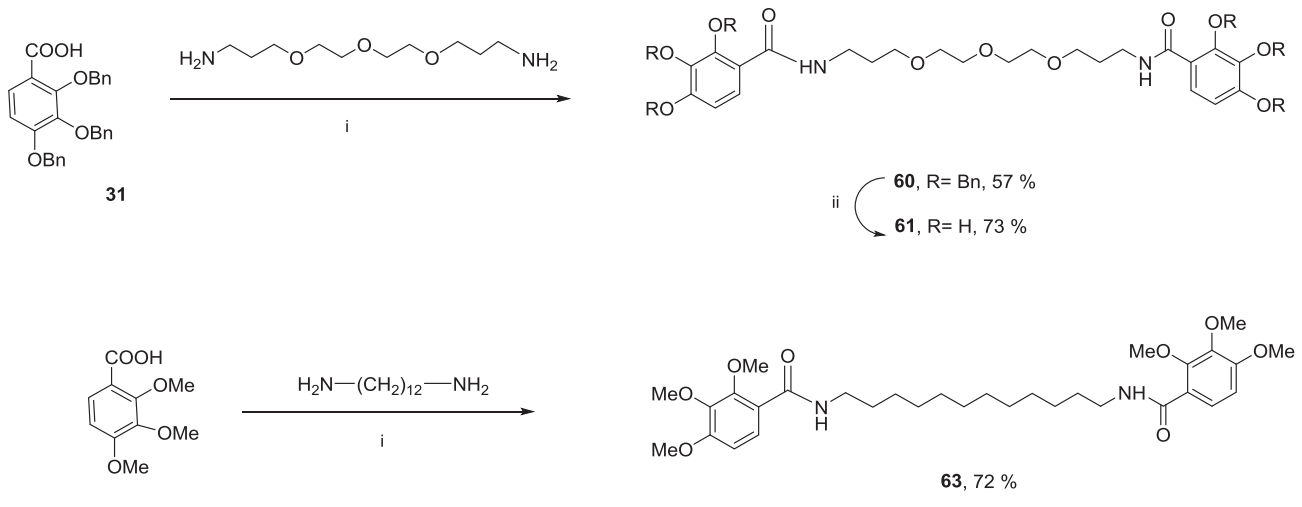
As it was shown for HIV none of the OMe- or OBn-protected compounds proved active at subtoxic concentrations (data not shown). These data point again to the importance of unsubstituted phenolic OHs as a requisite for antiviral activity. Table 2 summarizes the results of the anti-hepatitis C virus evaluation of the unprotected compounds.



Scheme 7. Synthesis of 2,3-dihydroxybenzoyl derivatives **50**–**52**. *Reagents and conditions*: (i) pyBOP, Et_3N (ii) BBr_3, CH_2Cl_2.



Scheme 8. Synthesis of 2-monohydroxybenzoyl derivatives **57**–**59**. *Reagents and conditions*: (i) pyBOP, Et_3N (ii) H_2, Pd/C.



Scheme 9. Synthesis of derivatives **61** and **63**. *Reagents and conditions*: (i) pyBOP, Et_3N (ii) H_2, Pd/C.

The "classical" alkyl gallate derivatives (**2**–**5**) showed activity against hepatitis C virus, albeit at concentrations that were close to the cytotoxic concentrations for the cells. Interestingly alkyl gallate **7**, with a 12 methylene linker, showed an activity (EC_{50} = 0.4 μM) far beneath the toxicity threshold (CC_{50} > 8.9), resulting in a selectivity index of >22.

With respect to the alkyl digallates **8**–**11** a tendency for the best efficiency is observed for compounds with longer alkyl chains. For instance, an increase in activity is observed when the alkyl length is expanded from C_4 (**8**) to C_{12} (**11**). The most active compound (**11**) has the highest anti-HCV selectivity (>7).

Among the alkyl amides of gallic acid **18**–**22** a relationship between the anti-hepatitis C virus activity and the number of carbons of the alkyl chain could not be found. Instead, a correlation between the length of the alkyl moiety and the anti-HCV activity was found for the amide derivatives **37**–**41**, containing two 2,3,4-trihydroxy benzoyl moieties at the two ends of the molecule, and **52** with two 2,3 -dihydroxy benzoyl moieties. Among these compounds, the best antiviral efficiency was observed for compounds **41** and **52** containing a C_{12} polymethylene linker. Compound **52** (EC_{50} = 2.0 μM; CC_{50} = 108) with two contiguous hydroxyl groups on the aromatic ring is the most selective of this series (selectivity

Table 1
Inhibitory effect of test compounds against HIV-1 and HIV-2 in CEM cell cultures.

	EC_{50} (μM)[a]		CC_{50} (μM)[b]
	HIV-1 (III_B)	HIV-2 (ROD)	
1	>50	>50	>250
2	>10	>10	23 ± 0
3	>10	>10	55 ± 43
4	14 ± 5	≥10	47 ± 38
5	5.4 ± 0.6	6.6 ± 1.1	14 ± 3
6	>2	>2	11 ± 8
7	≥1	≥1	2.8 ± 0.4
8	>10	>10	21 ± 3
9	≥10	≥10	22 ± 0.4
10	>10	>10	13 ± 7
11	4.6 ± 0.1	>10	4.7 ± 0.3
18	5.2 ± 0.9	>10	23 ± 1
19	>10	>10	72 ± 39
20	>10	–	51 ± 28
21	>50	>50	86 ± 12
22	19 ± 12	26 ± 2.1	58 ± 7.8
24	>10	>10	34 ± 14
26	18 ± 16	–	107 ± 0.7
28	10 ± 0.0	–	54 ± 33
30	4.9 ± 0.6	>10	21 ± 1.4
37	>10	>10	109 ± 87
38	>50	>50	102 ± 4.2
39	>50	>50	82 ± 11
40	>50	>50	100 ± 4.9
41	>10	>10	55 ± 21
43	2.6 ± 2.3	>10	27 ± 0.7
45	2.5 ± 1.9	>10	28 ± 9.3
50	>10	>10	19 ± 5.5
51	>2	–	5.2 ± 0.8
52	>2	>2	4.8
57	>10	>10	25 ± 2.8
58	>10	–	35 ± 3.5
59	>250	>250	248
61	>50	>50	81 ± 11
Tenofovir	3.9 ± 0.9	3.7 ± 1.2	>100
DS-5000	0.07 ± 0.02	0.03 ± 0.01	>20
Pradimicin A	3.3 ± 1.2	5.9 ± 3.7	>100

Data are the mean ± S.D. of at least 2 to 3 independent experiments.
[a] 50% Effective concentration, or compound concentration required to inhibit HIV-induced cytopathicity by 50%.
[b] 50% Cytostatic concentration, or compound concentration required to inhibit cell proliferation (CEM) by 50%.

Table 2
Antiviral effect of the test compounds on hepatitis C virus replication in the Huh 5–2 replicon system.

Compound	EC_{50} (μM)[a,d]	EC_{90} (μM)[b,d]	CC_{50} (μM)[c,d]
2	19	>45	45
3	13	>29	29
4	14	>50	>50
5	9.0	>50	>50
7	0.4	1.8	8.9
8	39	>50	>50
9	16	–	>50
10	17	55	80
11	6.5	37	>50
18	18	>29	29
19	11	>24	24
20	23	>50	>50
21	20	–	47
22	27	>100	>100
28	>2.9	>2.9	>2.9
30	34	>50	>50
37	15	>50	>50
38	14	–	32
39	25	–	34
40	11	–	>100
41	2.5	6.1	>100
43	34	>50	>50
45	>5.5	>5.5	>3.7
52	2.0	4.8	108
58	11	–	>49
59	4.7	>6.7	>6.7
61	28	–	49
63	>3.5	–	5.4
Telaprevir (VX-950)	2.8	–	47

[a] EC_{50} = 50% effective concentration (concentration at which 50% inhibition of virus replication is observed).
[b] EC_{90} = 90% effective concentration (concentration at which 90% inhibition of virus replication is observed).
[c] CC_{50} = 50% cytostatic/cytotoxic concentration (concentration at which 50% adverse effect is observed on the host cell).
[d] The EC_{50}, EC_{90} and CC_{50} values were determined in duplicate and with standard deviations of ±10% of the value quoted.

index: 54). Compound **41**, with three consecutive hydroxyl groups in the 2,3,4 position on the aromatic ring, also showed an activity (EC_{50} = 2.5 μM) far beneath the toxicity threshold (CC_{50} > 100), resulting in a selectivity index of >40. The comparable potency of both compounds indicates that the "extra" hydroxyl group present in **41** is not crucial for the activity.

Two related analogs of **41**, compounds **61** and **63**, have been prepared to assess the influence of the lipophilicity of the linking alkyl chain and the OH groups on the anti-HCV activity. The polyethylene glycol derivative **61** with a less lipophilic linker, was markedly less active than compound **41**, with a 12 methylene linker. No anti-HCV activity was observed with compound **63** with the phenolic OHs protected as OMe. This result clearly demonstrates that the free OH groups – alike HIV - play an important role in the anti-HCV activity and might also contribute to reduce cell toxicity.

Interestingly, alkyldigallamide **22** was less active than compound **41** revealing that the 2,3,4-trihydroxy phenyl moiety provides a better antiviral activity than the gallic acid moiety. These findings are consistent with previous observations made for HIV.

Compound **59**, containing a C_{12} polymethylene linker and only one single OH group on the terminal aromatic rings was only active at toxic concentrations. The inactivity of **59** (with only 1 OH on the aromatic ring) is in clear contrast with the activity shown by **41** and **52** (with 2 and 3 OHs on the aromatic ring) revealing that the presence of at least 2 hydroxyl groups in a contiguous position on the aromatic ring is important for antiviral activity/selectivity.

Branched compounds **28**, **43** and **45** with 3 galloyl or 2,3,4-trihydroxybenzoyl groups do not improve the antiviral efficacy of the linear compounds of equivalent length (**19**, **20**, **38** and **39**) with only two galloyl or 2,3,4-trihydroxybenzoyl groups, showing that the antiviral efficacy was not increased by the introduction of an extra (third) polyphenolic moiety. The same was observed for compound **30** bearing 5 galloyl groups, with respect to **21** and **22** of equivalent size.

In summary, with respect to the anti-hepatitis C virus activity, our structure-activity relationship studies clearly show that the activity seems to be related with the lipophilicity and hydroxyl substitution pattern. The most noticeable finding was the anti-hepatitis C virus activity shown by compounds **41** and **52** containing a 12 polymethylene chain and two 2,3 di- or 2,3,4 trihydroxybenzoyl groups located at the ends of the molecule, and leading to agents with a pronounced anti-HCV activity and selectivity. The "classical" alkyl gallate **7** also shows significant activity (six and five-fold higher than those of **41** and **52**, respectively) but its notorious toxicity makes it less attractive as a lead compound.

Compounds tested can inadvertently inhibit heterologous elements such as the encephalomyocarditis virus (EMCV) internal ribosome-entry site (IRES) or the neomycin phosphotransferase (neo) that are required for the proper translation of the HCV polyprotein in the assay system or to keep the HCV subgenomic replicon persistently in cells, respectively. Therefore we evaluated a

representative member of the compounds reported in this study, namely **41**, in different replicon systems. On the one hand we used Huh 9–13 HCV replicons that have the same genetic make-up as Huh 5–2 replicons, but differ in their composition of cell culture adaptive mutations, the absence of a luciferase reporter gene and the higher HCV RNA content as compared to Huh 5–2 cells [32]. Furthermore, we included a subgenomic replicon contained in HuH6 cells that again have a similar genetic make-up as Huh 5–2 replicons, but (*i*) replicate in a different cell line (HuH6 cells instead of Huh-7 cells) and (*ii*) carry different adaptive mutations. Moreover, in HCV subgenomic replicon containing HuH6 cells, replicon replication is not inhibited by IFN-γ and viral RNA replication is independent from cell proliferation [33].

Compound **41** proved to be active in both Huh 9–13 and HuH6 HCV subgenomic replicon containing cells at EC_{50}-values of 8.4 ± 1.6 μM and 33 μM, respectively, as determined by RT-qPCR. In both replicon containing cells **41** did not show any effect on the proliferation and metabolism of the host cell as determined by the MTS assay (CC_{50} > 100 μM) at the concentrations tested. The obtained values are in the same range as for the Huh 5–2 HCV subgenomic replicon containing cells. These findings exclude an inhibitory effect on the luciferase expressed by the HCV subgenomic replicons in Huh 5–2 cells and also show that inhibition is independent of the genetic make-up of the replicon and of the cell line used.

As reported previously HCV subgenomic replicon replication is highly dependent on cell proliferation [33]. Hence, any compound that can induce a cytostatic effect on cell growth could result indirectly in inhibition of HCV subgenomic replicon replication [34]. At the concentrations tested none of the compounds showed marked inhibition of cell growth, as apparent from the MTS assay (see Table 2). However, microscopic examination did highlight reduced cell numbers and altered cell morphology for most of the phenolic derivatives tested (as exemplified in Fig. 2 for **41**).

Furthermore HCV replication is highly dependent on the cellular lipid metabolism [35]. Any perturbation of the lipid metabolism, for example inhibition of fatty acid oxidation, might result indirectly in the inhibition of HCV replication [36]. Candidate drugs can perturb the lipid metabolism of a cell in different ways, for example by inducing phospholipidosis and steatosis, as reviewed in Ref. [37]. To assess if the compounds studied altered the cellular lipid metabolism we used Nile Red staining to assess the ability of compound **41** to induce phospholipidosis or drug induced steatosis.

Cells treated with a serial dilutions of compound **41** were analyzed using Nile red staining to visualize the intracellular accumulation of neutral lipids (hallmark of phospholipidosis) and charged lipids. Overall Nile Red staining showed that derivative **41** significantly reduced cell growth at concentrations between 33 and 100 μM. Furthermore these treated cells show a higher charged lipid content, as apparent of the increased cytoplasmic red stain, and an apparent rounded cell shape (Fig. 2, panels A, B) as compared to the mock-treated cells (Fig. 2, panel F). At lower concentrations (in the same range as the EC_{50} and EC_{90}-values) derivative **41** did not affect the size nor the number of the lipid droplets represented by the green dots in Nile Red stained cells and had no observable effect on cell number and cellular shape. These observations suggest that the alteration of cell morphology and charged lipid content of the cell by **41** at concentrations (≥33 μM) that are higher than those required to markedly inhibit HCV replication (EC_{50}: 2.5 μM).

All of these findings led us to conclude that compound **41** can be a useful tool to understand the biology of HCV infection. Alternatively it will be a useful prototype for the development of novel HCV therapeutics.

With respect to the mechanism of action of **41** it is intriguing to speculate what structural features (long polymethylene linker and two phenolic moieties at the end of the molecule) might contribute to its inhibitory action. As pointed out by several studies most of the bioactivities showed by the natural green tea catechins and synthetic alkyl gallates have been attributed to their antioxidant, free radical scavenging and metal chelating properties (iron, manganese, vanadium, copper, aluminum, calcium, etc …) [7c]. Another important aspect of these molecules that has special significance for their bioactivity is their amphipathic character. This property is due to the presence in their structures of hydrophobic aromatic rings and hydrophilic hydroxyls and allow them to establish interactions with biologically relevant molecules such as proteins and polysaccharides [7c-k].

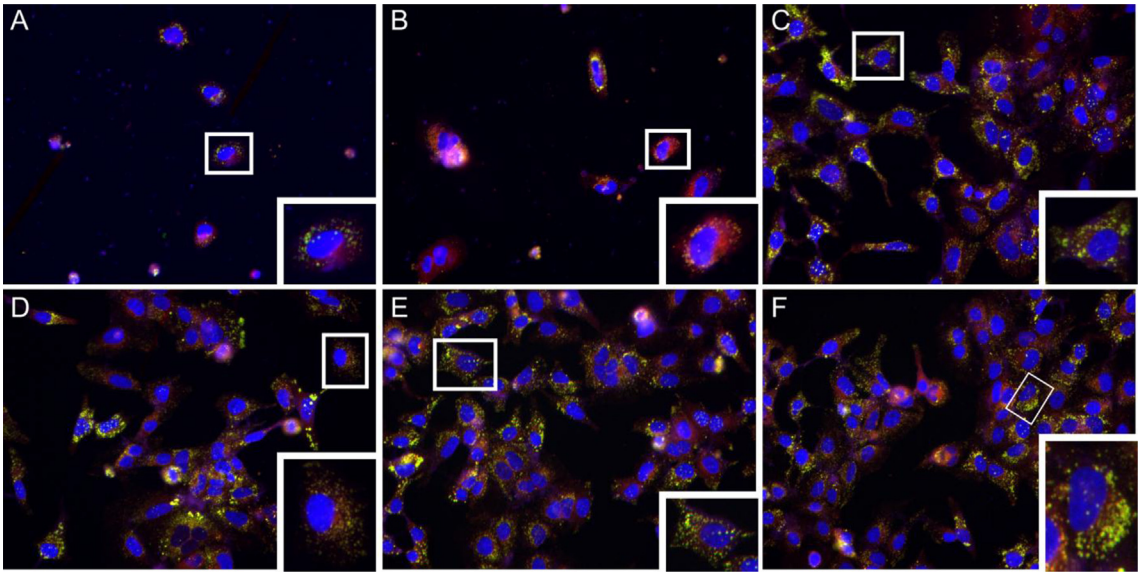


Fig. 2. Huh 9–13 cells were treated with different concentrations of derivative **41** (Panel A. 100 μM, B. 33 μM, C. 11 μM, D. 4 μM, E. 1 μM) or mock treated (Panel F) and stained with Nile Red. Boxes highlight cells that are magnified in the insert of each panel. Nile Red stains both neutral lipids (green color) and phospholipids (red color).

Taking into account the above general considerations we speculate that the compound **41** might inhibit various stages in the HCV life cycle by targeting key non-structural HCV proteins such as NS3/4A protease, NS5BRNA-dependent RNA polymerase and NS5A. In addition it might inhibit the HCV/host cell fusion process by targeting structural proteins on the HCV surface. Moreover the antioxidant properties of the phenolic rings present in **41** can affect host cell factors that are of importance in the replication of HCV.

3. Conclusions

We have synthesized different series of phenolic acid derivatives in which gallic acid (3,4,5-trihydroxybenzoic acid) and its isomer 2,3,4-trihydroxybenzoic acid, together with other mono- and dihydroxybenzoyl residues have been used as phenolic substituents and linear polyamines or polyalcohols as scaffolds. The compounds showed preferential activity against HIV and HCV.

In general, the presence of free phenolic OHs is of crucial importance for the antiviral activity since the compounds with the phenolic OHs protected as OMe or OBn were, as a rule, inactive against HIV and HCV.

With respect to the anti-HIV activity it was shown that tripodal compounds, with three phenolic moieties, showed higher activities than linear compounds with only one or two.

With respect to the anti-hepatitis C virus activity, the most notable finding was the selective anti-hepatitis C virus activity shown by compounds **41** and **52** containing a 12-polymethylene chain and two 2,3 di- or 2,3,4 tri-hydroxybenzoyl groups located at the ends of the molecule. At least two contiguous hydroxyl groups on the aromatic ring seem to be important for activity since the compounds with only one OH on the aromatic ring were inactive.

Interestingly, compounds bearing 2,3,4-trihydroxy benzoyl moieties were more active against both HIV and HCV than compounds substituted with galloyl groups. This observation led us to conclude that the 2,3,4-trihydroxyphenyl moiety provides better antiviral activities than the gallic acid moiety. This moiety might serve for the further rational exploration of compounds with anti-HCV/HIV properties.

Further studies are needed to develop derivatives with improved antiviral activity and to determine how these compounds inhibit HCV.

4. Materials and methods

4.1. Synthesis

4.1.1. Microwave assisted conditions for the synthesis of alkyl gallates *2*–*7*

A microwave vial was loaded with gallic acid **1** (0.3 mmol, 50 mg), the corresponding alcohol (0.9 mmol), and concentrated H_2SO_4 (0.07 mL). The reaction vessel was sealed and irradiated in a microwave reactor at 100 °C for 50 min. After cooling, volatiles were evaporated to dryness and the residue was dissolved in ethyl acetate (30 mL) and washed successively with saturated solutions of $NaHCO_3$ (3 × 20 mL) and NaCl (1 × 20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue was then purified by column chromatography using hexane/EtOAc, (3:7) as the eluent. Melting points and characterization of compounds **2**–**7** were consistent with those found in the literature [25d]. In our case, the following yields were obtained: **2** (70%), **3** (97%), **4** (83%), **5** (98%), **6** (96%), **7** (82%).

4.1.2. Tetramethylene-1,4-diyl-bis-(3,4,5-trihydroxybenzoate) (*8*)

Gallic acid **1** (3.3 mmol, 566 mg), 1,4-butanediol (1.1 mmol, 100 mg) and a catalytic amount of *p*-toluensulfonic acid were dissolved in dioxane (5 mL) in a distillation system. The mixture was heated at 160 °C distilling the solvent which is continuously refilled until the reaction was completed. The reaction mixture was allowed to reach room temperature and then was cooled by a water-ice bath to give a precipitate that was filtered and washed with gently quantities of diethyl ether to give 305 mg (70%) of **8** as a white solid; m.p. 221–222 °C. ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ 1.89 (m, 4H, CH_2), 4.30 (m, 4H, OCH_2), 7.14 (s, 4H, ArH), 8.07 (s, 2H, OH), 8.27 (s, 4H, OH). ^{13}C NMR [$(CD_3)_2CO$, 75 MHz] δ: 27.2 (CH_2), 65.7 (CH_2), 110.6 (ArCH), 122.6 (ArC), 139.7 (ArC), 147.0 (ArC), 168.0 (CO). MS (ES+): *m*/*z* 395.3 $(M+H)^+$. Anal. $C_{18}H_{18}O_{10}$ (C, H, N, O).

4.1.3. Hexamethylene-1,6-diyl-bis-(3,4,5-trihydroxybenzoate) (*9*)

Characterization of this compound in the literature was incomplete and NMR was registered in $CDCl_3$ [22b] and DMSO [22a]. Missing data (melting point) and NMR in acetone were now included.

In a procedure analogous to that described for compound **8**, gallic acid **1** (3.0 mmol, 510 mg) and 1,6-hexanediol (1.0 mmol, 118 mg) afforded 83 mg (28%) of **9** as a white solid; m.p. 212–215 °C. ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ 1.50 (m, 4H, CH_2), 1.74 (m, 4H, CH_2), 4.23 (t, J = 6.5 Hz, 4H, OCH_2), 7.14 (s, 4H, ArH), 8.22 (bs, OH). MS (ES+): *m*/*z* 423.1 $(M+H)^+$. Anal. $C_{20}H_{22}O_{10}$ (C, H, N, O).

4.1.4. Octamethylene-1,8-diyl-bis-(3,4,5-trihydroxybenzoate) (*10*)

Characterization of this compound in the literature was incomplete and NMR was registered in $CDCl_3$ [22b] and DMSO [22a]. Missing data (melting point) and NMR in acetone were now included.

In a procedure analogous to that described for compound **8**, gallic acid **1** (3.0 mmol, 510 mg) and 1,8-octanediol (1.0 mmol, 146 mg) afforded 221 mg (50%) of **10** as a white solid; m.p. 98–101 °C. ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ 1.50 (m, 8H, CH_2), 1.74 (m, 4H, CH_2), 4.21 (t, J = 6.5 Hz, 4H, OCH_2), 7.12 (s, 4H, ArH), 8.22 (bs, OH). ^{13}C NMR [$(CD_3)_2CO$, 75 MHz] δ: 26.8 (CH_2), 29.9(CH_2), 30.4(CH_2), 65.4 (CH_2), 110.3 (ArCH), 122.2 (ArC), 138.9 (ArC), 146.4 (ArC), 166.9 (CO). MS (ES+): *m*/*z* 451.2 $(M+H)^+$. Anal. $C_{22}H_{26}O_{10}$ (C, H, N, O).

4.1.5. Dodecamethylene-1,12-diyl-bis-(3,4,5-trihydroxybenzoate) (*11*)

Characterization of this compound in the literature was incomplete and NMR was registered in $CDCl_3$ [22b] and DMSO [22a]. Missing data (melting point) and NMR in acetone were now included.

In a procedure analogous to that described for compound **8**, gallic acid **1** (3.0 mmol, 510 mg), and 1,12-dodecanediol (1.0 mmol, 202.3 mg) afforded 264 mg (52%) of **11** as a white solid; m.p. 190–192 °C. ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ 1.40 (m, 16H, CH_2), 1.74 (m, 4H, CH_2), 4.21 (t, J = 6.5 Hz, 4H, OCH_2), 7.12 (s, 4H, ArH), 8.21 (bs, OH). ^{13}C NMR [$(CD_3)_2CO$, 75 MHz] δ: 26.15 (CH_2), 28.9 (CH_2), 29.4 (CH_2), 29.7 (CH_2), 33.0 (CH_2), 64.4 (CH_2), 109.1 (ArCH), 121.4 (ArC), 138.0 (ArC), 145.4 (ArC), 166.1 (CO). MS (ES+): *m*/*z* 507.0 $(M+H)^+$, 529.1 $(M + Na)^+$. Anal. $C_{26}H_{34}O_{10}$ (C, H, N, O).

4.2. General procedure for the synthesis of alkyl amides *18*–*22*, *24*, *26* and *30*

3,4,5-Trimethoxybenzoyl chloride **12** (3 eq) and potassium carbonate (3 eq) were dissolved in a mixture of ethyl acetate/water (1:1) and treated with the corresponding amine (1 eqv). The resulting biphasic solution was stirred overnight and a white precipitate appeared. The solid (OMe protected derivative) was filtered and the filtrate was dried and subsequently dissolved in dry

dichloromethane. The resulting solution was cooled by a water-ice bath (0–5 °C) and treated, under argon atmosphere, with boron tribromide (1.5 eq for each OMe group). The reaction was left to reach room temperature and stirred overnight. The precipitate that appeared was filtered and washed repeatedly with gently quantities of cold dichloromethane and ether to give the corresponding unprotected derivative as a white solid. Eventually some products had to be purified by reversed phase SPE cartridges using water/methanol (20:1 → 1:1) as the eluent.

4.2.1. *N,N′-tetramethylenebis(3,4,5-trihydroxybenzamide) (**18**)*

1,4-Tetramethylenediamine (75 mg, 0.88 mmol), potassium carbonate (364.87 mg, 2.64 mmol) and 3,4,5-trimethoxybenzoyl chloride (**12**) (609 mg, 2.64 mmol) reacted according to the general procedure to give 380 mg (91%) of the OMe protected derivative **13** as a white solid; m.p. 243–244 °C.^{1}H NMR [$(CD_3)_2SO$, 300 MHz] δ 1.55 (m, 4H, CH_2), 3.67 (m, 4H, CH_2NH), 3.80 (s, 18H, OCH_3), 7.1 (s, 4H, ArH), 8.42 (t, $J = 5.5$ Hz, 2H, NH). MS (ES+): m/z 477.0 $(M+H)^+$, 499.3 $(M + Na)^+$. Anal. $C_{24}H_{32}N_2O_8$ (C, H, N, O).

Compound **13** (200 mg, 0.21 mmol) was treated with boron tribromide (0.12 mL) obtaining 62.3 mg (76%) of **18** as a white solid; m.p. 238–240 °C.^{1}H NMR [$(CD_3)_2SO$, 300 MHz] δ 1.46 (bs, 4H, CH_2), 3.16 (bs, 4H, CH_2NH), 6.80 (s, 4H, ArH), 8.05 (bs, 2H, NH); 8.83 (bs, 6H, OH). ^{13}C NMR [$(CD_3)_2SO$, 75 MHz] δ: 27.2 (CH_2), 39.3 (CH_2), 107.0 (ArCH), 125.5 (ArC), 136.4 (ArC), 145.7 (ArC), 166.7 (CO). MS (ES+): m/z 393.1 $(M+H)^+$. Anal. $C_{18}H_{20}N_2O_8$ (C, H, N, O).

4.2.2. *N,N′-hexamethylenebis(3,4,5-trihydroxybenzamide) (**19**)*

1,6-Hexamethylenediamine (75 mg, 0.64 mmol), potassium carbonate (266.74 mg, 1.93 mmol) and 3,4,5-trimethoxybenzoyl chloride (**12**) (445 mg, 1.93 mmol) reacted according to the general procedure to give 314 mg (98%) of the OMe protected derivative **14** as a white solid; m.p. 219–221 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.45 (m, 4H, CH_2), 1.64 (m, 4H, CH_2), 3.45 (c, $J = 6.7$ Hz, $J = 6.2$ Hz, 4H, CH_2NH), 3.87 (s, 6H, OCH_3), 3.90 (s, 12H, OCH_3), 6.13 (t, $J = 5.5$ Hz, 2H, NH), 6.99 (s, 4H, ArH). MS (ES+): m/z 505.3 $(M+H)^+$. Anal. $C_{26}H_{36}N_2O_8$ (C, H, N, O).

Compound **14** (200 mg, 0.40 mmol) was treated with boron tribromide (0.28 mL) obtaining 45.2 mg (51%) of **19** as a white solid. Melting point and spectroscopic characterization of compound **19** were consistent with those found in the literature [38].

4.2.3. *N,N′-octamethylenebis(3,4,5-trihydroxybenzamide) (**20**)*

1,8-Octamethylenediamine (100 mg, 1.13 mmol), potassium carbonate (468.5 mg, 3.39 mmol) and 3,4,5-trimethoxybenzoyl chloride (**12**) (780 mg, 3.39 mmol) reacted according to the general procedure to obtain 464 mg (77%) of the OMe protected derivative **15** as a white solid; m.p. 201–202 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.37 (m, 8H, CH_2), 1.63 (m, 4H, CH_2), 3.43 (c, $J = 6.9$ Hz, $J = 6.5$ Hz, 4H, CH_2NH), 3.87 (s, 18H, OCH_3), 6.43 (t, $J = 5.1$ Hz, 2H, NH), 7.06 (s, 4H, ArH). MS (ES+): m/z 533.1 $(M+H)^+$. Anal. $C_{28}H_{40}N_2O_8$ (C, H, N, O).

Compound **15** (77 mg, 0.14 mmol) was treated with boron tribromide (0.08 mL) obtaining 54.3 mg (84%) of **20** as a white solid; m.p.155–157 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ 1.38 (bs, 8H, CH_2), 1.58 (m, 4H, CH_2), 3.34 (m, 4H, CH_2NH), 6.82 (s, 4H, ArH), MS (ES+): m/z 449.6 $(M+H)^+$. Anal. $C_{22}H_{28}N_2O_8$ (C, H, N, O).

4.2.4. *N,N′-decamethylenebis(3,4,5-trihydroxybenzamide) (**21**)*

1,10-Decamethylenediamine (100 mg, 0.58 mmol), potassium carbonate (240.5 mg, 1.74 mmol) and 3,4,5-trimethoxybenzoyl chloride (402 mg, 1.74 mmol) reacted according to the general procedure to obtain 179 mg (65%) of the OMe protected derivative **16** as an amorphous white solid; ^{1}H NMR [$(CD_3)_2SO$, 400 MHz] δ 1.18–1.30 (m, 12H, CH_2), 1.45–1.53 (m, 4H, CH_2), 3.10–3.23 (m, 4H, CH_2NH), 3.65 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.74–3.80 (m, 9H, OCH_3), 3.84 (s, 3H, OCH_3), 7.14 (s, 4H, ArH), 8.35 (bs, 2H, NH). ^{13}C NMR [$(CD_3)_2SO$, 100 MHz] δ 23.82 (CH_2), 27.15 (CH_2), 29.39 (CH_2), 29.59 (CH_2), 29.86 (CH_2), 56.85 (CH_3), 105.77 (ArCH), 107.67 (ArCH), 130.63 (ArC), 153.22 (ArC), 165.99 (CO), 167.52 (CO). MS (ES+): m/z 561.2 $(M+H)^+$. Anal. $C_{30}H_{44}N_2O_8$ (C, H, N, O).

Compound **16** (100 mg, 0.18 mmol) was treated with boron tribromide (0.102 mL) obtaining 60.1 mg (70%) of **21** as a white solid; m.p. 126–128 °C. ^{1}H NMR [CD_3OD, 300 MHz] δ 1.24–1.35 (m, 12H, CH_2), 1.49–1.62 (m, 4H, CH_2), 3.22–3.32 (m, 4H, CH_2NH, overlaps with CD_3OD), 6.83 (s, 2H, ArH). ^{13}C NMR [CD_3OD, 75 MHz] δ 28.55 (CH_2), 30.08 (CH_2), 30.99 (CH_2), 32.90 (CH_2), 41.35 (CH_2), 108.14 (ArCH), 108.96 (ArCH), 126.71 (ArC), 146.88 (ArC), 173.57 (CO). MS (ES+): m/z 477.5 $(M+H)^+$. Anal. $C_{24}H_{32}N_2O_8$ (C, H, N, O).

4.2.5. *N,N′-dodecamethylenebis(3,4,5-trihydroxybenzamide) (**22**)*

1,12-Dodecamethylenediamine (100 mg, 0.50 mmol), potassium carbonate (207.3 mg, 1.50 mmol) and 3,4,5-trimethoxybenzoyl chloride (345 mg, 1.50 mmol) reacted according to the general procedure to obtain 206 mg (70%) of the OMe protected derivative **17** as an amorphous white solid; ^{1}H NMR [$CDCl_3$, 400 MHz] δ 1.29 (m, 16H, CH_2), 1.55 (m, 4H, CH_2), 3.28 (m, 4H, CH_2NH), 3.85 (s, 6H, OCH_3), 3.88 (s, 12H, OCH_3), 6.95 (s, 4H, ArH). ^{13}C NMR [$CDCl_3$, 100 MHz] δ 27.11 (CH_2), 29.44 (CH_2), 29.59 (CH_2), 29.88 (CH_2), 40.32 (CH_2), 56.43 (CH_3), 61.23 (CH_3), 104.33 (ArCH), 130.77 (ArC), 141.37 (ArC), 153.63 (ArC), 167.35 (CO). MS (ES+): m/z 589.0 $(M+H)^+$. Anal. $C_{32}H_{48}N_2O_8$ (C, H, N, O).

Compound **17** (100 mg, 0.17 mmol) was treated with boron tribromide (0.097 mL) obtaining 62.5 mg (70%) of **22** as a white solid. Characterization of this compound in the literature [22b] was incomplete and NMR was made in DMSO-d_6. Missing data (melting point) and NMR in acetone were now included; m.p. 135–137 °C. ^{1}H-RMN [$(CD_3)_2SO$, 300 MHz] δ 1.24 (m, 16H, CH_2), 1.45 (m, 4H, CH_2), 3.16 (m, 4H, CH_2NH), 6.80 (s, 4H, ArH), 8.00 (t, $J = 5.5$ Hz, 2H, NH), 8.25 (bs, OH). ^{13}C NMR [$(CD_3)_2SO$, 75 MHz] δ: 26.9 (CH_2), 29.2 (CH_2), 29.4 (CH_2), 29.6 (CH_2), 31.0 (CH_2), 39.4 (CH_2), 107.0 (ArCH), 125.5 (ArC), 136.4 (ArC), 145.7 (ArC), 166.6 (CO). MS (ES+): m/z 505.0 $(M+H)^+$. Anal. $C_{26}H_{36}N_2O_8$ (C, H, N, O).

4.2.6. *N,N′,N″-tris-(3,4,5-trihydroxybenzoyl)diethylenetriamine (**24**)*

Diethylenetriamine (100 mg, 0.96 mmol), potassium carbonate (531 mg, 3.84 mmol) and 3,4,5-trimethoxybenzoyl chloride (**12**) (708 mg, 3.07 mmol) reacted according to the general procedure to give 241 mg (38%) of the OMe protected derivative **23** as a white solid; m.p. 96–99 °C. ^{1}H NMR [($CDCl_3$, 400 MHz] δ 3.50 (s, 6H, OCH_3), 3.55–3.68 (m, 4H, CH_2NH), 3.78–3.91 (m, 25H, CH_2NH, OCH_3), 6.29 (s, 2H, ArH), 7.05–7.13 (m, 4H, ArH), 7.53 (bs, NH). ^{13}C NMR $CDCl_3$, 100 MHz] δ: 39.37 (CH_2), 40.06 (CH_2), 45.57 (CH_2), 51.00 (CH_2), 55.95 (CH_3), 56.39 (CH_3), 61.09 (CH_3), 103.43 (ArCH), 104.23 (ArCH), 104.74 (ArCH), 129.16 (ArC), 131.28 (ArC), 138.59 (ArC), 140.93 (ArC), 153.50 (ArC), 173.75 (CO). MS (ES+): m/z 686.1 $(M+H)^+$. Anal. $C_{34}H_{43}N_3O_{12}$ (C, H, N, O).

Compound **23** (548 mg, 0.8 mmol) was treated with boron tribromide (0.70 mL) obtaining 130.5 mg (30%) of **24** as an amorphous white solid. ^{1}H NMR [D_2O, 300 MHz] δ 3.16–3.54 (m, 8H, CH_2NH), 6.01 (bs, 2H, NH), 6.52 (bs, 3H, ArH), 6.62 (bs, 3H, ArH). MS (ES+): m/z 560.2 $(M+H)^+$. Anal. $C_{25}H_{25}N_3O_{12}$ (C, H, N, O).

4.2.7. *N,N′,N″-tris(3,4,5-trihydroxybenzoyl)spermidine (**26**)*

Spermidine (50 mg, 0.34 mmol), potassium carbonate (188 mg, 1.36 mmol) and 3,4,5-trimethoxybenzoyl chloride (**12**) (313 mg, 1.36 mmol) reacted according to the general procedure to give 96.3 mg (39%) of the OMe protected derivative **25** as a white solid; m.p. 78–80 °C. ^{1}H NMR [$CDCl_3$, 400 MHz] δ 1.40–1.68 (m, 4H, CH_2),

1.81–1.99 (m, 2H, C*H*$_2$–CH$_2$–NH), 3.22–3.67 (m, 8H, C*H*$_2$NH), 3.78 (s, 9H, OCH$_3$), 3.83 (s, 9H, OCH$_3$), 3.84 (s, 9H, OCH$_3$), 6.49 (bs, 2H, ArH), 7.00 (bs, 2H, ArH), 7.17 (bs, 2H, ArH), 7.85 (bs, 2H, NH). ^{13}C NMR [(CDCl$_3$, 100 MHz] δ: 26.55 (CH$_2$), 27.34 (CH$_2$), 27.72 (CH$_2$), 37.13 (CH$_2$), 39.64 (CH$_2$), 42.46 (CH$_2$), 49.38 (CH$_2$), 53.67 (CH$_3$), 56.46 (CH$_3$), 61.06 (CH$_3$), 61.10 (CH$_3$), 103.86 (ArCH), 104.56 (ArCH), 104.64 (ArCH), 129.88 (ArC), 131.94 (ArC), 139.19 (ArC), 140.86 (ArC), 141.11 (ArC), 153.28 (ArC), 153.37 (ArC), 153.57 (ArC), 167.15 (CO), 167.48 (CO), 172.66 (CO). MS (ES+): *m/z* 728.3 (M+H)$^+$. Anal. C$_{37}$H$_{49}$N$_3$O$_{12}$ (C, H, N, O).

Compound **25** (72.7 mg, 0.1 mmol) was treated with boron tribromide (0.085 mL) obtaining 56.7 mg (95%) of **26** as a white solid. ^{1}H NMR (CD$_3$OD, 300 MHz) δ 1.76 (m, 6H, CH$_2$), 2.99 (m, 8H, C*H*$_2$–NH), 6.41 (bs, 3H, ArH), 6.85 (bs, 3H, ArH). MS (ES+): *m/z* 602.0 (M+H)$^+$. Anal. C$_{28}$H$_{31}$N$_3$O$_{12}$ (C, H, N, O).

4.2.8. Tris[2-(3′,4′,5′-trihydroxybenzamido)ethyl]amine (**28**)

Tris(2-aminoethyl)amine (50 mg, 0.34 mmol), potassium carbonate (188 mg, 1.36 mmol) and 3,4,5-trimethoxybenzoyl chloride (**12**) (313 mg, 1.36 mmol) reacted according to the general procedure to give 171.8 mg (72%) of the OMe protected derivative **27** as a white solid. Characterization of this compound in the literature was incomplete [29] and ^{1}H NMR was made in CDCl$_3$. Missing data (melting point) and ^{1}H NMR in acetone were now included; m.p. 150–152 °C. ^{1}H NMR [(CD$_3$)$_2$CO, 300 MHz] δ 2.77 (t, *J* = 5.6 Hz, 6H, CH$_2$N), 3.52 (m, 6H, C*H*$_2$NH), 3.72 (s, 9H, OCH$_3$), 3.76 (s, 18H, OCH$_3$), 7.17 (s, 6H, ArH), 7.98 (t, *J* = 5.1 Hz, 3H, NH). ^{13}C NMR [(CDCl$_3$, 100 MHz] δ: 35.46 (CH$_2$), 56.47 (CH$_3$), 57.91 (CH$_2$), 61.00 (CH$_3$), 105.07 (ArCH), 127.77 (ArC), 141.36 (ArC), 153.20 (ArC), 167.54 (CO). MS (ES+): *m/z* 729.1 (M+H)$^+$. Anal. C$_{36}$H$_{48}$N$_4$O$_{12}$ (C, H, N, O).

Compound **27** (107 mg, 0.15 mmol) was treated with boron tribromide (0.13 mL) obtaining 82 mg (93%) of **28** as a white solid. Melting point and spectroscopic characterization of compound **28** were consistent with those found in the literature [29].

4.2.9. N,N′,N″,N‴,N⁗-pentakis(3,4,5-trihydroxybenzoyl) tetraethylenepentamine (**30**)

Tetraethylenepentamine (50 mg, 0.26 mmol) potassium carbonate (215.6 mg, 1.56 mmol) and 3,4,5-trimethoxybenzoyl chloride (**12**) (359 mg, 1.56 mmol) reacted according to the general procedure to give 153.3 mg (85%) of the OMe protected derivative **29** as a white solid; m.p. 102–105 °C. ^{1}H NMR [(CD$_3$)$_2$CO, 300 MHz] δ 3.40–3.90 (m, 61H, CH$_2$, OCH$_3$), 6.33–7.09 (m, 10H, ArH), 8.06 (bs, NH). ^{1}H NMR [(CDCl$_3$, 400 MHz] δ 3.40–3.90 (m, 61H, CH$_2$, OCH$_3$), 6.33–7.09 (m, 10H, ArH), 8.06 (bs, NH). MS (ES+): *m/z* 1161.2 (M+H)$^+$. Anal. C$_{58}$H$_{73}$N$_5$O$_{20}$ (C, H, N, O).

Compound **29** (105 mg, 0.09 mmol) was treated with boron tribromide (0.13 mL) obtaining **30** quantitatively as a white solid; m.p. 175–177 °C. ^{1}H NMR (CD$_3$OD, 400 MHz) δ 3.21–3.92 (m, 16H, CH$_2$), 6.20–6.56 (m, 6H, ArH), 6.76–6.98 (m, 4H, ArH). ^{13}C NMR [(CD$_3$OD, 100 MHz] δ: 55.06 (CH$_2$), 55.52 (CH$_2$), 102.79 (ArCH), 104.81 (ArCH), 106.03 (ArCH), 106.47 (ArCH), 106.72 (ArCH), 108.70 (ArCH), 124.13 (ArC), 125.57 (ArC), 135.10 (ArC), 137.30 (ArC), 137.93 (ArC), 145.25 (ArC), 145.50 (ArC), 145.89 (ArC), 148.13 (ArC), 148.46 (ArC), 169.86 (CO), 174.25 (CO). MS (ES+): *m/z* 950.1 (M+H)$^+$. Anal. C$_{43}$H$_{43}$N$_5$O$_{20}$ (C, H, N, O).

4.3. General method for the synthesis of alkyl amides **37**–**41**, **43** and **45**

Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (pyBOP) (1.1 mmol for each NH moiety) was added to a solution of 2,3,4-tribenzyloxybenzoic acid **31** (1.1 mmol for each NH moiety) in dry dichloromethane (20 mL). After 5 min, triethylamine (TEA) (1 mmol for each NH moiety) and the corresponding amine (1 mmol) were added. The mixture was stirred at room temperature overnight and then evaporated to dryness. The residue was dissolved in ethyl acetate (30 mL) and washed successively with saturated solutions of citric acid (3 × 20 mL), NaHCO$_3$ (3 × 20 mL) and NaCl (1 × 20 mL). The residue was concentrated and purified by column chromatography using hexane/ethyl acetate (5:1).

A solution of the corresponding OBn protected derivative in THF/MeOH (1:1) containing 30 wt% of Pd/C (10%) was hydrogenated at 30 °C overnight under atmospheric pressure using a reaction balloon filled with hydrogen gas and a glass flask as the reaction vessel. The Pd/C was filtered through Whatman® filter paper 42 and the solvent was removed under reduced pressure to give the crude product which was then purified by triturating with diethyl ether or dichloromethane to afford the corresponding deprotected derivative.

4.3.1. N,N′-tetramethylenebis(2,3,4-hydroxybenzamide) (**37**)

According to the general procedure 1,4-tetramethylenediamine (35.2 mg, 0.40 mmol) was treated with 2,3,4-tribenzyloxybenzoic acid **31** (440 mg, 1.00 mmol) to give 373 mg (100%) of the OBn protected derivative **32** as a white solid; m.p. 145–147 °C. ^{1}H NMR [CDCl$_3$, 300 MHz] δ 1.18–1.25 (m, 4H, CH$_2$), 3.15–3.20 (m, 4H, C*H*$_2$NH), 5.07 (s, 4H, C*H*$_2$Ph), 5.11 (s, 4H, C*H*$_2$Ph), 5.17 (s, 4H, C*H*$_2$Ph), 6.89 (d, 2H, *J* = 9.6 Hz, ArH), 7.30–7.50 (m, 30H, ArH), 7.92 (d, 2H, *J* = 9.6 Hz, ArH). Anal. C$_{60}$H$_{56}$N$_2$O$_8$ (C, H, N, O).

Following the deprotection procedure, the OBn derivative **32** (100 mg, 0.107 mmol) gave a crude product which was then purified by triturating with diethyl ether to afford 19.1 mg (46%) of **37** as a pale yellow solid; m.p. 225–227 °C. ^{1}H NMR [CD$_3$OD, 500 MHz] δ 1.60–1.78 (m, 4H, CH$_2$), 3.35–3.50 (m, 4H, C*H*$_2$NH), 6.34 (d, 2H, *J* = 9.2 Hz, ArH), 7.12 (d, 2H, *J* = 9.2 Hz, ArH). ^{13}C NMR [CD$_3$OD, 125 MHz] δ 26.57 (CH$_2$), 38.56 (CH$_2$), 106.37 (ArC), 107.47 (ArC), 117.63 (ArC), 132.56 (ArC), 149.21 (ArC), 150.22 (ArC), 170.47 (CO). MS (ES+): *m/z* 393.0 (M+H)$^+$. Anal. C$_{18}$H$_{20}$N$_2$O$_8$ (C, H, N, O).

4.3.2. N,N′-hexamethylenebis(2,3,4-hydroxybenzamide) (**38**)

According to the general procedure 1,6-hexamethylenediamine (23,3 mg, 0.20 mmol) was treated with 2,3,4-tribenzyloxybenzoic acid **31** (220 mg, 0.50 mmol) to give 131.3 mg (69%) of the OBn protected derivative **33** as a white solid; m.p. 134–135 °C. ^{1}H NMR [CDCl$_3$, 300 MHz] δ 1.05–1.17 (m, 4H, CH$_2$), 1.17–1.32 (m, 4H, CH$_2$), 3.16–3.27 (m, 4H, C*H*$_2$NH), 5.08 (s, 4H, C*H*$_2$Ph), 5.13 (s, 4H, C*H*$_2$Ph), 5.16 (s, 4H, C*H*$_2$Ph), 6.88 (d, 2H, *J* = 9.3 Hz, ArH), 7.28–7.51 (m, 30H, ArH), 7.92 (d, 2H, *J* = 9.3 Hz, ArH). ^{13}C NMR [CDCl$_3$, 75 MHz] δ 26.99 (CH$_2$), 29.49 (CH$_2$), 39.95 (CH$_2$), 71.46 (C*H*$_2$Ph), 76.06 (C*H*$_2$Ph), 109.73 (ArCH), 120.12 (ArC), 127.99 (ArCH), 128.62 (ArCH), 128.82 (ArCH), 129.06 (ArCH), 129.08 (ArCH), 129.16 (ArCH), 132.46 (ArC), 133.57 (ArC), 136.67 (ArC), 136.73 (ArC), 137.57 (ArC), 141.61 (ArC), 152.07 (ArC), 156.05 (ArC), 165.15 (CO). Anal. C$_{62}$H$_{60}$N$_2$O$_8$ (C, H, N, O).

Following the deprotection procedure, the OBn derivative **33** (121.66 mg, 0.126 mmol) gave a crude product which was then purified by triturating with diethyl ether to give 22 mg (42%) of **38** as a pale yellow solid; m.p. 163–165 °C. ^{1}H NMR [CD$_3$OD, 300 MHz] δ 1.40–1.49 (m, 4H, CH$_2$), 1.56–1.69 (m, 4H, CH$_2$), 3.30–3.40 (m, 4H, C*H*$_2$NH, overlaps with CD$_3$OD), 6.35 (d, 2H, *J* = 8.8 Hz, ArH), 7.13 (d, 2H, *J* = 8.8 Hz, ArH). ^{13}C NMR [CD$_3$OD, 75 MHz] δ 26.86 (CH$_2$), 29.45 (CH$_2$), 39.45 (CH$_2$), 106.82 (ArC), 107.95 (ArCH), 117.94 (ArCH), 133.02 (ArC), 149.52 (ArC), 150.48 (ArC), 170.74 (CO). MS (ES+): *m/z* 421.4 (M+H)$^+$. Anal. C$_{20}$H$_{24}$N$_2$O$_8$ (C, H, N, O).

4.3.3. N,N′-octamethylenebis(2,3,4-hydroxybenzamide) (**39**)

According to the general procedure 1,8-octamethylenediamine (28.8 mg, 0.20 mmol) was treated with 2,3,4-tribenzyloxybenzoic

acid **31** (220 mg, 0.50 mmol) to give 121.9 mg (62%) of the OBn protected derivative **34** as a white solid; m.p. 122–124 °C. ^{1}H NMR [$CDCl_3$, 300 MHz] δ 1.09–1.23 (m, 8H, CH_2), 1.23–1.35 (m, 4H, CH_2), 3.19–3.28 (m, 4H, CH_2NH), 5.09 (s, 4H, CH_2Ph), 5.12 (s, 4H, CH_2Ph), 5.17 (s, 4H, CH_2Ph), 6.89 (d, 2H, J = 9.1 Hz, ArH), 7.26–7.49 (m, 30H, ArH), 7.86 (bs, 1H, NH), 7.93 (d, 2H, J = 9.1 Hz, ArH). ^{13}C NMR [$CDCl_3$, 75 MHz] δ 27.56 (CH_2), 29.59 (CH_2), 29.72 (CH_2), 39.95 (CH_2), 71.29 (CH_2Ph), 76.23 (CH_2Ph), 109.67 (ArCH), 120.35 (ArC), 127.31 (ArCH), 128.00 (ArCH), 128.61 (ArCH), 128.81 (ArCH), 129.05 (ArCH), 129.09 (ArCH), 129.13 (ArCH), 136.67 (ArC), 136.81 (ArC), 137.55 (ArC), 141.46 (ArC), 152.17 (ArC), 156.02 (ArC), 165.14 (CO). Anal. $C_{64}H_{64}N_2O_8$ (C, H, N, O).

Following the deprotection procedure, the OBn derivative **34** (112.2 mg, 0.113 mmol) gave a crude product which was then purified by triturating with dichloromethane to afford 25 mg (48%) of **39** as a white solid; m.p. 179–181 °C. ^{1}H NMR [CD_3OD, 300 MHz] δ 1.36–1.44 (m, 8H, CH_2), 1.55–1.67 (m, 4H, CH_2), 3.27–3.36 (m, 4H, CH_2NH, overlaps with CD_3OD), 6.35 (d, 2H, J = 8.8 Hz, ArH), 7.12 (d, 2H, J = 8.8 Hz, ArH). ^{13}C NMR [CD_3OD, 75 MHz] δ 27.98 (CH_2), 30.33 (CH_2), 30.50 (CH_2), 40.36 (CH_2), 107.79 (ArC), 108.98 (ArCH), 119.05 (ArCH), 133.57 (ArC), 150.71 (ArC), 151.54 (ArC), 171.76 (CO). MS (ES+): m/z 449.6 $(M+H)^+$. Anal. $C_{22}H_{28}N_2O_8$ (C, H, N, O).

4.3.4. *N,N′-decamethylenebis(2,3,4-hydroxybenzamide) (**40**)*

According to the general procedure 1,10-diaminodecane (34.5 mg, 0.20 mmol) was treated with 2,3,4-tribenzyloxybenzoic acid (220 mg, 0.50 mmol) to give 97.4 mg (51%) of the OBn protected derivative **35** as a white solid; m.p. 110–112 °C. ^{1}H NMR [$CDCl_3$, 300 MHz] δ 1.17–1.23 (m, 12H, CH_2), 1.25–1.35 (m, 4H, CH_2), 3.21–3.29 (m, 4H, CH_2NH), 5.08 (s, 4H, CH_2Ph), 5.13 (s, 4H, CH_2Ph), 5.17 (s, 4H, CH_2Ph), 6.89 (d, 2H, J = 9.2 Hz, ArH), 7.28–7.48 (m, 30H, ArH), 7.92 (d, 2H, J = 9.2 Hz, ArH). ^{13}C NMR [$CDCl_3$, 75 MHz] δ 27.35 (CH_2), 29.72 (CH_2), 29.89 (CH_2), 39.82 (CH_2), 71.30 (CH_2Ph), 76.01 (CH_2Ph), 109.60 (ArCH), 120.18 (ArC), 127.30 (ArCH), 127.99 (ArCH), 128.61 (ArCH), 128.81 (ArCH), 129.11 (ArCH), 136.68 (ArC), 136.75 (ArC), 137.51 (ArC), 141.75 (ArC), 152.22 (ArC), 156.03 (ArC), 165.17 (CO). Anal. $C_{66}H_{68}N_2O_8$ (C, H, N, O).

Following the deprotection procedure, the OBn derivative **35** (90 mg, 0.088 mmol) gave a crude product which was then purified by triturating with dichloromethane to afford 25.3 mg (61%) of **40** as a white solid; m.p. 177–179 °C. ^{1}H NMR [CD_3OD, 300 MHz] δ 1.28–1.39 (m, 12H, CH_2), 1.52–1.64 (m, 4H, CH_2), 3.27–3.39 (m, 4H, CH_2NH, overlaps with CD_3OD), 6.35 (d, 2H, J = 9.1 Hz, ArH), 7.12 (d, 2H, J = 9.1 Hz, ArH). ^{13}C NMR [CD_3OD, 75 MHz] δ 28.02 (CH_2), 30.35 (CH_2), 30.50 (CH_2), 30.54 (CH_2), 40.36 (CH_2), 107.77 (ArC), 108.96 (ArCH), 119.03 (ArCH), 133.96 (ArC), 150.57 (ArC), 151.56 (ArC), 171.74 (CO). MS (ES+): m/z 477.5 $(M+H)^+$. Anal. $C_{24}H_{32}N_2O_8$ (C, H, N, O).

4.3.5. *N,N′-dodecamethylenebis(2,3,4-hydroxybenzamide) (**41**)*

According to the general procedure 1,12-dodecamethylendiamine (80.14 mg, 0.40 mmol) was treated with 2,3,4-tribenzyloxybenzoic acid (440 mg, 1 mmol) to give 417 mg (100%) of the OBn protected derivative **36** as a white solid; m.p. 92–94 °C. ^{1}H NMR [$CDCl_3$, 300 MHz] δ 0.90–1.00 (m, 4H, CH_2), 1.17–1.40 (m, 16H, CH_2), 3.20–3.40 (m, 4H, CH_2–NH), 5.07 (s, 4H, CH_2Ph), 5.14 (s, 4H, CH_2Ph), 5.19 (s, 4H, CH_2Ph), 6.80–7.00 (m, 2H, ArH), 7.28–7.51 (m, 28H, ArH), 7.80–8.00 (m, 4H, ArH). Anal. $C_{68}H_{72}N_2O_8$ (C, H, N, O).

Following the deprotection procedure, the OBn derivative **36** (106 mg, 0.102 mmol) gave a crude product which was then purified by triturating with dichloromethane to afford 24 mg (47%) of **41** as a white solid; m.p. 151–153 °C. ^{1}H NMR [CD_3OD, 500 MHz] δ 1.20–1.47 (m, 16H, CH_2), 1.52–1.58 (m, 4H, CH_2), 3.30–3.40 (m, 4H, CH_2NH), 6.34 (d, 2H, J = 8.8 Hz, ArH), 7.12 (d, 2H, J = 8.8 Hz, ArH). ^{13}C NMR [CD_3OD, 75 MHz] δ 28.54 (CH_2), 30.89 (CH_2), 31.01 (CH_2), 31.12 (CH_2), 31.14 (CH_2), 40.89 (CH_2), 108.27 (ArC), 109.47 (ArC), 119.54 (ArC), 134.49 (ArC), 151.08 (ArC), 152.09 (ArC), 172.25 (CO). MS (ES+): m/z 505.3 $(M+H)^+$. Anal. $C_{26}H_{36}N_2O_8$ (C, H, N, O).

4.3.6. *N,N′,N″-tris(2,3,4-trihydroxybenzoyl)spermidine (**43**)*

According to the general procedure spermidine (50 mg, 0.34 mmol) was treated with 2,3,4-tribenzyloxybenzoic acid **31** (500 mg, 1.13 mmol) to give 240 mg (75%) of the OBn protected derivative **42** as a white solid; m.p. 68–70 °C. ^{1}H NMR [$CDCl_3$, 300 MHz] δ 1.00–1.20 (m, 1H, CH_2), 1.30–1.42 (m, 2H, CH_2), 1.43–1.54 (m, 2H, CH_2), 1.60–1.76 (m, 1H, CH_2), 2.80–3.00 (m, 1H, CH_2), 3.02–3.20 (m, 4H, CH_2), 3.20–3.40 (m, 2H, CH_2), 3.60–3.80 (m, 1H, CH_2), 4.96–5.30 (m, 18H, CH_2Ph), 6.70–6.96 (m, 5H, ArH), 7.20–7.60 (m, 42H, ArH), 7.70–8.10 (m, 4H, ArH). Anal. $C_{91}H_{85}N_3O_{12}$ (C, H, N, O).

Following the deprotection procedure, the OBn derivative **42** (128.7 mg, 0.09 mmol) gave a crude product which was then purified by triturating with diethyl ether to afford 23 mg (42%) of **43** as a white solid; m.p. 185–187 °C. ^{1}H NMR [CD_3OD, 500 MHz] δ 1.30–1.60 (m, 4H, CH_2), 1.70–1.80 (m, 2H, CH_2), 3.25–3.62 (m, 8H, CH_2NH), 6.22–6.31 (m, 3H, ArH), 6.43 (d, 1H, J = 8.3 Hz, ArH), 7.01 (d, 2H, J = 8.3 Hz, ArH). ^{13}C NMR [CD_3OD, 125 MHz] δ 26.30 (CH_2), 28.10 (CH_2), 36.03 (CH_2), 38.37 (CH_2), 48.20 (CH_2), 54.62 (CH_2), 106.39 (ArC), 106.51 (ArC), 106.82 (ArC), 107.34 (ArC), 107.47 (ArC), 115.67 (ArC), 117.55 (ArC), 117.60 (ArC), 117.64 (ArC), 132.54 (ArC), 132.56 (ArC), 132.94 (ArC), 143.02 (ArC), 147.04 (ArC), 149.22 (ArC), 149.26 (ArC), 150.20 (ArC), 150.24 (ArC), 170.41 (CO), 170.43 (CO), 171.81 (CO). MS (ES+): m/z 602.2 $(M+H)^+$. Anal. $C_{28}H_{31}N_3O_{12}$ (C, H, N, O).

4.3.7. *Tris[2-(2′,3′,4′-trihydroxybenzamide)ethyl]amine (**45**)*

According to the general procedure tris(2-aminoethyl)amine (0.052 mL, 0.34 mmol) was treated with 2,3,4-tribenzyloxybenzoic acid **31** (494 mg, 1.12 mmol) to give 478 mg (100%) of the OBn protected derivative **44** as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.28–2.36 (m, 6H, CH_2N), 3.08–3.21 (m, 6H, CH_2NH), 4.98–5.20 (m, 18H, CH_2Ph), 6.70–6.86 (m, 3H, ArH), 7.22–7.43 (m, 42H, ArH), 7.75–7.87 (m, 3H, ArH).^{13}C NMR [$CDCl_3$, 75 MHz] δ 36.92 (CH_2), 70.77 (CH_2), 76.01 (CH_2), 77.22 (CH_2), 109.28 (ArC), 120.12 (ArC), 126.93 (ArC), 127.78 (ArC), 128.45 (ArC), 128.63 (ArC), 128.86 (ArC), 128.91 (ArC), 129.02 (ArC), 136.39 (ArC), 136.53 (ArC), 137.32 (ArC), 141.28 (ArC), 152.02 (ArC), 155.92 (ArC), 175.13 (CO). MS (ES+): m/z 1413.1 $(M+H)^+$. Anal. $C_{90}H_{84}N_4O_{12}$ (C, H, N, O).

Following the deprotection procedure, the OBn derivative **44** (220 mg, 0.15 mmol) gave a crude product which was then purified by triturating with diethyl ether to afford 32 mg (35%) of **45** as a white solid. Characterization of this compound in the literature was incomplete [29c] and NMR was made in D_2O.. Missing data (melting point) and NMR in methanol were now included; m.p. 154–156 °C.^{1}H NMR (CD_3OD, 300 MHz) δ 2.83 (t, J = 6.20 Hz, 6H, CH_2N), 3.49 (t, J = 6.12 Hz, 6H, CH_2NH), 6.26 (d, J = 8.80 Hz, 3H, ArH), 7.03 (d, J = 8.81 Hz, 3H, ArH). MS (ES+): m/z 603.7 $(M+H)^+$. Anal. $C_{27}H_{30}N_4O_{12}$ (C, H, N, O).

4.4. *General method for the synthesis of alkyl amides **50**–**52** and **57**–**59***

Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (pyBOP) (2.2 mmol) was added to a solution of 2,3-dimethoxybenzoic acid **46** (2.2 mmol) or 2-benzyloxysalicylic acid **53** (2.2 mmol) in dry dichloromethane (20 mL). After 5 min, triethylamine (TEA) (2 mmol) and the corresponding diamine (1 mmol) were added. The mixture was stirred at room temperature overnight and then evaporated to dryness. The residue was

dissolved in ethyl acetate (30 mL) and washed successively with saturated solutions of citric acid (3 × 20 mL), $NaHCO_3$ (3 × 20 mL) and NaCl (1 × 20 mL). The residue was concentrated and purified by preparative centrifugal circular thin-layer chromatography (CCTLC) using dichloromethane/methanol (9:1 → 1:1) as the eluent.

The protected OMe derivatives **47**–**49** were deprotected using boron tribromide as was previously described to afford the deprotected derivatives **50**–**52**. Melting points and characterization of these compounds were consistent with those found in the literature [31]. In our case the following total yields were obtained: **50** (81%), **51** (99%) and **52** (50%).

Deprotection of the benzyl groups of **54**–**56** by catalytic hydrogenation in the presence of 10% Pd/C as was previously described afforded the corresponding phenol deprotected derivatives **57** (74%), **58** (99%) and **59** (50%). Melting points and characterization of these compounds were consistent with those found in the literature [31a,39].

4.4.1. *N,N′-4,7,10-trioxa-1,13-tridecanebis(2,3,4-hydroxybenzamide) (**61**)*

Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (pyBOP) (0.37 mmol) was added to a solution of 2,3,4-tribenzyloxybenzoic acid **31** (163.7 mg, 0.37 mmol) in dry dichloromethane (5 mL). After 5 min, triethylamine (TEA) (0.37 mmol) and 4,7,10-trioxa-1,13-tridecanediamine (35.24 mg, 0.16 mmol) were added. The mixture was stirred at room temperature overnight and then evaporated to dryness. The residue was dissolved in ethyl acetate (20 mL) and washed successively with saturated solutions of citric acid (3 × 20 mL), $NaHCO_3$ (3 × 20 mL) and NaCl (1 × 20 mL). The residue was concentrated and purified by preparative centrifugal circular thin-layer chromatography (CCTLC) using hexane/ethyl acetate (1:1) as eluent to give 96.5 mg (57%) of the OBn protected derivative **60** as a colorless oil. ^{1}H NMR [$CDCl_3$, 300 MHz] δ 1.50–1.60 (m, 4H, CH_2), 3.35 (q, 4H, J = 6.4 Hz, CH_2O), 3.35 (t, 4H, J = 5.8 Hz, CH_2NH), 3.43–3.49 (m, 4H, CH_2O), 3.51–3.56 (m, 4H, CH_2O), 4.97 (s, 4H, CH_2Ph), 5.02 (s, 8H, CH_2Ph), 6.74 (d, 2H, J = 8.7 Hz, ArH), 7.19–7.38 (m, 30H, ArH), 7.77 (d, 2H, J = 8.7 Hz, ArH), 7.92 (t, 2H, J = 5.5 Hz, NH).^{13}C NMR [$CDCl_3$, 75 MHz] δ 29.70 (CH_2), 36.80 (CH_2), 69.12 (CH_2), 70.09 (CH_2), 70.28 (CH_2), 71.27 (CH_2Ph), 76.10 (CH_2Ph), 109.47 (ArCH), 119.82 (ArCH), 128.00 (ArCH), 128.81 (ArCH), 129.03 (ArCH), 129.18 (ArCH), 136.64 (ArC), 137.60 (ArC), 141.34 (ArC), 152.16 (ArC), 156.17 (ArC), 165.55 (CO). Anal. $C_{66}H_{68}N_2O_{11}$ (C, H, N, O).

Following the deprotection procedure, the OBn derivative **60** (89.6 mg, 0.084 mmol) gave a crude product which was coevaporated with diethyl ether to give 40 mg (73%) of **61** as a brownish oil. ^{1}H NMR [CD_3OD, 300 MHz] δ 1.83 (q, 4H, J = 6.4 Hz, CH_2), 3.29–3.64 (m, 16H, CH_2O, CH_2NH), 6.36 (d, 2H, J = 8.8 Hz, ArH), 7.11 (d, 2H, J = 8.8 Hz, ArH). ^{13}C NMR [CD_3OD, 75 MHz] δ 30.61 (CH_2), 38.25 (CH_2), 70.72 (CH_2), 71.41 (CH_2), 71.66 (CH_2), 108.25 (ArC), 109.95 (ArCH), 119.47 (ArCH), 134.03 (ArC), 150.96 (ArC), 151.93 (ArC), 172.06 (CO). MS (ES+): m/z 525.5 $(M+H)^+$. Anal. $C_{24}H_{32}N_2O_{11}$ (C, H, N, O).

4.4.2. *N,N′-dodecamethylenebis(2,3,4-trimethoxybenzamide) (**63**)*

In a procedure analogous to that described for compound **60**, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (pyBOP) (0.5 mmol) and 2,3,4-trimethoxybenzoic acid **62** (106.1 mg, 0.5 mmol) in dry dichloromethane (5 mL) was treated with triethylamine (TEA) (0.5 mmol) and 1,12-dodecamethylendiamine (34.46 mg, 0.20 mmol) to obtain 85.2 mg (72%) of **63** as a white solid; m.p. 62–64 °C. ^{1}H NMR [$CDCl_3$, 300 MHz] δ 1.18–1.41 (m, 16H, CH_2), 1.50–1.62 (m, 4H, CH_2), 3.40 (q, 4H, J = 6.6 Hz, CH_2NH), 3.82 (s, 6H, CH_3), 3.85 (s, 6H, CH_3), 3.91 (s, 6H, CH_3), 6.72 (d, 2H, J = 9.5 Hz, ArH), 7.84 (d, 2H, J = 9.5 Hz, ArH), 7.87–7.93 (m, 2H, NH). ^{13}C NMR [$CDCl_3$, 75 MHz] δ 27.31 (CH_2), 29.52 (CH_2), 29.75 (CH_2), 29.83 (CH_2), 39.79 (CH_2), 56.21 (CH_3), 61.13 (CH_3), 107.73 (ArC), 119.35 (ArC), 126.79 (ArC), 141.88 (ArC), 152.48 (ArC), 156.40 (ArC), 164.97 (CO). MS (ES+): m/z 589.1 $(M+H)^+$. Anal. $C_{32}H_{48}N_2O_8$ (C, H, N, O).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.01.033.

References

[1] I. Saito, T. Miyamura, A. Obayashi, H. Harada, T. Katayama, S. Kikuchi, Y. Watanabe, S. Koi, M. Onji, Y. Ohata, Q.-L. Choo, M. Houghton, G. Kuo, Hepatitis C virus infection is associated with the development of hepatocellular carcinoma, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 6547–6549.

[2] (a) M.W. Fried, M.L. Shiffman, K.R. Reddy, C. Smith, G. Marinos, F.L. Gonçales, D. Häussinger, M. Diago, G. Carosi, D. Dhumeaux, A. Craxi, A. Lin, J. Hoffman, J. Yu, Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection, N. Engl. J. Med. 347 (2002) 975–982;
(b) M.G. Ghany, D.R. Nelson, D.B. Strader, D.L. Thomas, L.B. Seeff, An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American association for the study of liver diseases, Hepatol. Baltim. Md. 54 (2011) 1433–1444;
(c) EASL clinical practice Guidelines, management of hepatitis C virus infection, J. Hepatol. 55 (2011) 245–264.

[3] (a) T.J. Liang, M.G. Ghany, Current and future therapies for hepatitis C virus infection, N. Engl. J. Med. 368 (2013) 1907–1917;
(b) P.J. Pockros, Drugs in development for chronic hepatitis C: a promising future, Expert Opin. Biol. Ther. 11 (2011) 1611–1622;
(c) C. Sarrazin, C. Hezode, S. Zeuzem, J.M. Pawlotsky, Antiviral strategies in hepatitis C virus infection, J. Hepatol. 56 (Suppl. 1) (2012) S88–S100;
(d) C.A.M. Stedman, Current prospects for interferon-free treatment of hepatitis C in 2012, J. Gastroenterol. Hepatol. 28 (1) (2013) 38–45.

[4] (a) I.M. Jacobson, J.G. McHutchison, G. Dusheiko, A.M. Di Bisceglie, K.R. Reddy, N.H. Bzowej, P. Marcellin, A.J. Muir, P. Ferenci, R. Flisiak, J. George, M. Rizzetto, D. Shouval, R. Sola, R.A. Terg, E.M. Yoshida, N. Adda, L. Bengtsson, A.J. Sankoh, T.L. Kieffer, S. George, R.S. Kauffman, S. Zeusem, Telaprevir for previously untreated chronic hepatitis C virus infection, N. Engl. J. Med. 364 (2011) 2405–2416;
(b) F. Poordad, J. McCone Jr., B.R. Bacon, S. Bruno, M.P. Manns, M.S. Sulkowski, I.M. Jacobson, K.R. Reddy, Z.D. Goodman, N. Boparai, M.J. DiNubile, V. Sniukiene, C.A. Brass, J.K. Albrecht, J.-P. Bronowicki, Boceprevir for untreated chronic HCV genotype 1 infection, N. Engl. J. Med. 364 (2011) 1195–1206;
(c) C. Scheridan, New Merck and Vertex drugs raise standard of care in hepatitis C, Nat. Biotechnol. 29 (2011) 553–554;
(d) J.G. McHutchison, G.T. Everson, S.C. Gordon, I.M. Jacobson, M. Sulkowski, R. Kauffman, L. McNair, J. Alam, A.J. Muir, Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection, N. Engl. J. Med. 360 (2009) 1827–1838;
(e) C. Hezode, N. Forestier, G. Dusheiko, P. Ferenci, S. Pol, T. Goeser, J.-P. Bronowicki, M. Bourlière, S. Gharakhanian, L. Bengtsson, L. McNair, S. George, T. Kieffer, A. Kwong, R.S. Kauffman, J. Alam, J.-M. Pawlotsky, S. Zeuzem, Telaprevir and peginterferon with or without ribavirin for chronic HCV infection, N. Engl. J. Med. 360 (2009) 1839–1850;
(f) B.R. Bacon, S.C. Gordon, E. Lawitz, P. Marcellin, J.M. Vierling, S. Zeuzem, F. Poordad, Z.D. Goodman, H.L. Sings, N. Boparai, M. Burroughs, C.A. Brass, J.K. Albrecht, R. Esteban, Boceprevir for previously treated chronic HCV genotype 1 infection, N. Engl. J. Med. 364 (2011) 1207–1217.

[5] (a) J.M. Pawlotsky, Treatment failure and resistance with direct-acting antiviral drugs against hepatitis C virus, Hepatology 53 (2011) 1742–1751;
(b) S. Susser, C. Welsch, Y. Wang, M. Zettler, F.S. Domingues, U. Karey, E. Hughes, R. Ralston, X. Tong, E. Herrmann, S. Zeuzem, C. Sarrazin, Characterization of resistance to the protease inhibitor boceprevir in hepatitis C virus-infected patients, Hepatology 50 (2009) 1709–1718.

[6] (a) R.J. Pomerantz, D.L. Horn, Twenty years of therapy for HIV-1 infection, Nat. Med. 9 (7) (2003) 867–873;
(b) P.A. Volberding, S.G. Deeks, Antiretroviral therapy and management of HIV infection, Lancet 376 (9734) (2010) 49–62;
(c) C. Flexner, HIV drug development: the next 25 years, Nat. Rev. Drug. Discov. 6 (2007) 959–966.

[7] (a) D. Ferreira, J.P. Steynberg, J.P. Roux, E.V. Brandt, Diversity of structure and function in oligomeric flavanoids, Tetrahedron 48 (1992) 1743–1803;
(b) D. Ferreira, R. Bekker, Oligomeric proanthocyanidins: naturally occurring O-heterocycles, Nat. Prod. Rep. 13 (1996) 411–433;
(c) J.G. Handique, J.B. Baruah, Polyphenolic compounds: an overview, React. Funct. Polym. 52 (2002) 163–188;
(d) J. Harborne, Plant polyphenols, in: E. Bell, B. Charlwood (Eds.), Encyclopedia of Plant Physiology, Springer Verlag, Berlin Heidelberg New York, 1980, pp. 329–395;
(e) E. Haslam, Y. Cai, Plant polyphenols (vegetable tannins): gallic acid metabolism, Nat. Prod. Rep. 11 (1994) 41–65;
(f) E. Haslam, Practical Polyphenolics-from Structure to Molecular Recognition and Physiological Action, Cambridge University Press, Cambridge, 1998;
(g) R.W. Hemingway, P.E. Laks (Eds.), Plant Polyphenols. Synthesis, Properties, Significance, Plenum, New York, London, 1992;
(h) S. Quideau, K.S. Feldman, Ellagitannin chemistry, Chem. Rev. 96 (1996) 475–503;
(i) S. Quideau, D. Deleux, C. Douat-Casassus, L. Pousegu, Plant polyphenols: chemical properties, biological activities, and synthesis, Chem. Int. Ed. 50 (2011) 586–621;
(j) E. Haslam, Natural polyphenols (vegetable tannins) as drugs: possible modes of action, J. Nat. Prod. 59 (1996) 205–215;
(k) P. Sarni-Manchado, J.-M. Canals-Bosh, G. Mazerolles, V. Cheynier, Influence of the glycosylation of human salivary proline-rich proteins on their interactions with condensed tannins, J. Agric. Food. Chem. 56 (2008) 9563–9569.

[8] (a) A. Ali, F. Alqurainy (Eds.), Activities of Antioxidants in Plants under Environmental Stress, Transworld Research Network, 2006;
(b) M.M. Caldwell, R. Robberecht, S.D. Flint, Internal filters: prospects for UV-acclimation in higher plants, Physiol. Plant 58 (1983) 445–450;
(c) H.A. Stafford, K. Ibrahim (Eds.), Phenolic Metabolism in Plants, Plenum, New York, 1992.

[9] I. Mueller-Harvey, Unravelling the conundrum of tannins in animal nutrition and health, J. Sci. Food. Agric. 86 (1992) 2010–2037.

[10] (a) I.E. Dreosti, Bioactive ingredients: antioxidants and polyphenols in tea, Nutr. Rev. 54 (1996) S51–S58;
(b) N. Khan, H. Mukhtar, Tea polyphenols for health promotion, Life Sci. 81 (2007) 519–533;
(c) N. Taylor, Green Tea, the Natural Secret for a Healthier Life, Kensington Publishing Corp, New York, N.Y., 2007;
(d) H. Wang, G.J. Provan, K. Helliwell, Tea flavonoids, their functions, utilization and analysis, Trends Food Sci. Tech. 11 (2000) 152–160.

[11] (a) J.V. Higdon, B. Frei, Tea catechins and polyphenols: health effects, metabolism and antioxidant functions, Crit. Rev. Food Sci. Nutr. 43 (1) (2003) 89–143;
(b) S.V. Jovanovic, S. Steenken, M. Tosic, B. Marjanovic, M.G. Simic, Flavonoids as antioxidants, J. Am. Chem. Soc. 116 (1994) 4846–4851;
(c) C. Cabrera, R. Artacho, R. Giménez, Beneficial effects of green tea—a review, J. Am. Coll. Nutr. 2 (2006) 79–99;
(d) I. Urquiaga, F. Leighton, Plant polyphenol antioxidants and oxidative stress, Biol. Res. 33 (2000) 55–64.

[12] (a) R. Cooper, D.J. Morré, D.M. Morré, Medicinal benefits of green tea: part II. Review of anticancer properties, J. Altern. Complement. Med. 11 (2005) 639–652;
(b) N. Khan, H. Mukhtar, Multitargeted therapy of cancer by green tea polyphenols, Cancer Lett. 269 (2) (2008) 269–280;
(c) S.N. Nichenametla, T.G. Taruscio, D.L. Barney, J.H. Exon, A review of the effects and mechanisms of polyphenolics in cancer, Crit. Rev. Food Sci. Nutr. 46 (2) (2006) 161–183;
(d) S. Shankar, S. Ganapathy, R.K. Srivastava, Green tea polyphenols: biology and therapeutic implications in cancer, Front. Biosci. 12 (2007) 4881–4899;
(e) C.S. Yang, X. Wang, G. Lu, S.C. Picinich, Regulation of innate immune responses in the brain, Nat. Rev. Cancer 9 (2009) 429–439;
(f) C.S. Yang, H. Wang, Mechanistic issues concerning cancer prevention by tea catechins, Mol. Nutr. Food Res. 55 (6) (2011) 819–831;
(g) N.T. Zaveri, Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications, Life Sci. 78 (18) (2006) 2073–2080.

[13] (a) A. Basu, E.A. Lucas, Mechanisms and effects of green tea on cardiovascular health, Nutr. Rev. (8 Pt 1) (2007) 361–375;
(b) P.V. Babu, D. Liu, Green tea catechins and cardiovascular health: an update, Curr. Med. Chem. 15 (18) (2008) 1840–1850;
(c) A. Deka, J.A. Vita, Tea and cardiovascular disease, Pharmacol. Res. 64 (2) (2011) 136–145;
(d) M.A. Islam, Cardiovascular effects of green tea catechins: progress and promise, Recent Pat. Cardiovasc. Drug Discov. 7 (2) (2012) 88–99;
(e) N. Jochmann, G. Baumann, V. Stangl, Green tea and cardiovascular disease: from molecular targets towards human health, Curr. Opin. Clin. Nutr. Metab. Care 11 (6) (2008) 758–765;
(f) A.B. Pon Velayutham, D. Liu, Green Tea catechins and cardiovascular health: an update, Curr. Med. Chem. 15 (18) (2008) 1840–1850;
(g) S. Wolfram, Effects of green tea and EGCG on cardiovascular and metabolic health, J. Am. Coll. Nutr. 26 (4) (2007) 373S–388S.

[14] (a) S. Mandel, M.B. Youdim, Catechin polyphenols: neurodegeneration and neuroprotection in neurodegenerative diseases, Free Radic. Biol. Med. 37 (3) (2004) 304–317;
(b) S.A. Mandel, T. Amit, O. Weinreb, M.B. Youdim, Understanding the broad-spectrum neuroprotective action profile of green tea polyphenols in aging and neurodegenerative diseases, J. Alzheimers Dis. 25 (2) (2011) 187–208;
(c) T. Pan, J. Jankovic, W. Le, Potential therapeutic properties of green tea polyphenols in Parkinson's disease, Drugs Aging 20 (10) (2003) 711–721.

[15] (a) M. Daglia, Polyphenols as antimicrobial agents, Curr. Opin. Biotechnol. 23 (2012) 174–181;
(b) M. Friedman, Overview of antibacterial, antitoxin, antiviral and antifungal activities of tea flavonoids and teas, Mol. Nutr. Food Res. 51 (2007) 116–134;
(c) J.M. Hamilton-Miller, Antimicrobial properties of tea (Camellia sinensis L.), Antimicrob. Agents Chemother. 39 (1995) 2375–2377;
(d) E. Haslam, Natural polyphenols (vegetable tannins) as drugs: possible modes of action, J. Nat. Prod. 59 (1996) 205–215;
(e) P.W. Taylor, J.M.T. Hamilton-Miller, P.D. Stapleton, Antimicrobial properties of green tea catechins, Food Sci. Technol. Bull. 2 (2005) 71–81;
(f) T.S. Yam, S. Shat, J.M. Hamilton-Miller, Microbiological activity of whole and fractionated crude extracts of tea (*Camellia sinensis*), and of tea components, FEMS Microbiol. Lett. 152 (1) (1997) 169–174.

[16] T.P. Tim, A. Cushnie, J. Lamb, Recent advances in understanding the antibacterial properties of flavonoids, Int. J. Antimicrob. Agents 38 (2) (2011) 99–107.

[17] M. Hirasawa, K. Takada, Multiple effects of green tea catechin on the antifungal activity of antimycotics against Candida albicans 53 (2) (2004) 225–229.

[18] (a) L.-K.- Chang, T.T. Wei, Y.F. Chiu, C.P. Tung, J.Y. Chuang, S.K. Hung, C. Li, S.T. Liu, Inhibition of Epstein-Barr virus lytic cycle by (-)-epigallocatechin gallate, Biochem. Biophys. Res. Commun. 301 (2003) 1062–1068;
(b) G. Fassina, A. Buffa, R. Benelli, O.E. Varnier, D.M. Noonan, A. Albini, Polyphenolic antioxidant (-)-epigallocatechin-3-gallate from green tea as a candidate anti-HIV agent, AIDS 16 (2002) 939–941;
(c) A. Hamza, C.G. Zhan, How can (-)epigallocatechin gallate from green tea prevent HIV-1 infection? J. Phys. Chem. B 110 (2006) 2910–2917;
(d) H.Y. Ho, M.L. Cheng, S.F. Weng, Y.L. Leu, D.T. Chin, Antiviral effect of epigallocatechin gallate on enterovirus 71, J. Agric. Food Chem. 52 (2009) 6140–6147;
(e) C.E. Isaacs, W. Xu, G. Merz, S. Hillier, I. Roham, G.Y. Wen, Digallate dimers of (-)-Epigallocatechin gallate inactivate herpes simplex virus, Antimicrob. Agents Chemother. 55 (2011) 5646–5653;
(f) F. Jiang, W. Chen, K. Yi, Z. Wu, Y. Si, W. Han, Y. Zhao, The evaluation of catechins that contain a galloyl moiety as potential HIV-1 integrase inhibitors, Clin. Immunol. 137 (2010) 347–356;
(g) K. Kawai, N.H. Tsuno, J. Kitayama, Y. Okaji, K. Yazawa, M. Asakage, N. Hori, T. Watanabe, K. Takahashi, H. Nagawa, Epigallocatechin gallate, the main component of tea polyphenol, binds to CD4 and interferes with gp120 binding, J. Allergy Clin. Immunol. 112 (2003) 951–957;
(h) J. Li, L. Zhou, Y. Zhang, Studies on the effects of tea catechins against hepatitis B virus infection, Zhonghua Yu Fang. Yi Xue Za Zhi 35 (2001) 404–407;
(i) Y.-T. Lin, Y.-H. Wu, C.-K. Tseng, C.-K. Lin, W.-C. Chen, Y.-C. Hsu, J.-C. Lee, Green tea phenolic epicatechins inhibit hepatitis C virus replication via cyclooxygenase-2 and attenuate virus-induced inflammation, Plos One 8 (2013) 1–10;
(j) S. Liu, H. Lu, Q. Zhao, Y. He, J. Niu, A.K. Debhath, S. Wu, S. Jiang, Theaflavin derivatives in black tea and catechin derivatives in green tea inhibit HIV-1 entry by targeting gp41, Biochim. Biophys. Acta 1723 (2005) 270–281;
(k) C.L. Nance, E.B. Siwak, W.T. Shearer, Preclinical development of the green tea catechin, epigallocatechin gallate, as an HIV-1 therapy, J. Allergy. Clin. Immunol. 123 (2009) 459–465;
(l) M. Nakayama, K. Suzuki, M. Toda, S. Okubo, Y. Hara, T. Shimamura, Preclinical development of the green tea catechin, epigallocatechin gallate, as an HIV-1 therapy, Antivir. Res. 21 (1993) 289–299;
(m) J.-M. Song, K.-H. Lee, B.-L. Seong, Antiviral effect of catechins in green tea on influenza virus, Antivir. Res. 68 (2) (2005) 66–74;
(n) J.M. Weber, A. Ruzindana-Umunyana, I. Imbeault, S. Sircar, Inhibition of adenovirus infection and adenain by green tea catechins, Antivir. Res. 58 (2) (2003) 168–173;
(o) M.P. Williamson, T.G. McCormick, C.L. Nance, W.T. Shearer, Epigallocatechin gallate, the main polyphenol in green tea, binds to the T-cell receptor, CD4: potential for HIV-1 therapy, J. Allergy Clin. Immunol. 118 (6) (2006) 1369–1374;
(p) K. Yamaguchi, M. Honda, H. Ikigai, Y. Hara, T. Shimamura, Inhibitory effects of (-)-epigallocatechin gallate on the life cycle of human immunodeficiency virus type 1 (HIV-1), Antivir. Res. 53 (1) (2002) 19–34;
(q) Ch E. Isaacs, G.Y. Wen, X. Weimin, J.H. Jia, L. Rohan, Ch Corbo, V. Di Maggio,

E.C. Jenkins Jr., S. Hillier, Epigallocatechin gallate inactivates clinical isolates of Herpes Simplex Virus, Antimicrob. Agents Chemother. 52 (3) (2002) 962–970;
(r) Y. Zhao, F. Jiang, P. Liu, W. Chen, K. Yi, Catechins containing a galloyl moiety as potential anti-HIV-1 compounds, Drug Discov. Today 17 (11–12) (2012) 630–635.

[19] M.M. Silva, M.R. Santos, G. Caroco, R. Rocha, G. Justino, L. Mira, Structure-antioxidant activity relationships of flavonoids: a re-examination, Free Radic. Res. 36 (11) (2002) 1219–1227.

[20] T. Taguri, T. Tanaka, I. Kouno, Antibacterial spectrum of plant polyphenols and extracts depending upon hydroxyphenyl structure, Biol. Pharm. Bull. 29 (11) (2006) 2226–2235.

[21] (a) I. Kubo, N. Masuoka, P. Xiao, H. Haraguchi, Antioxidant activity of dodecyl gallate, J. Agric. Food. Chem. 50 (2002) 3533–3539;
(b) C.A. Van der Heijden, P.J. Janssen, J.J. Strik, Toxicology of gallates: a review and evaluation, Food Chem. Toxicol. 24 (10–11) (1986) 1067–1070.

[22] (a) K. Dodo, T. Minato, T. Noguchi-Yachide, M. Suganuma, Y. Hashimoto, Antiproliferative and apoptosis-inducing activities of alkyl gallate and gallamide derivatives related to (-)-epigallocatechin gallate, Bioorg. Med. Chem. 16 (2008) 7975–7982;
(b) K. Dodo, T. Minato, Y. Hashimoto, Structure–Activity relationship of bis-galloyl derivatives related to (-)-Epigallocatechin gallate, Chem. Pharm. Bull. 57 (2) (2009) 190–194;
(c) S.M. Fiuza, C. Gomes, L.J. Teixeira, M.T. Girão da Cruz, M.N.D.S. Cordeiro, N. Milhazes, F. Borges, M.P.M. Marques, Phenolic acid derivatives with potential anticancer properties––a structure–activity relationship study. Part 1: methyl, propyl and octyl esters of caffeic and gallic acids, Bioorg. Med. Chem. 12 (2004) 3581–3589;
(d) C. Frey, M. Pavani, G. Cordano, S. Muñoz, E. Rivera, J. Medina, A. Morello, J. Diego Maya, J. Ferreira, Comparative cytotoxicity of alkyl gallates on mouse tumor cell lines and isolated rat hepatocytes, Comp. Biochem.. Physiol. A Mol. Integr. Physiol. 146 (4) (2006) 520–527;
(e) R. Veluri, R.P. Singh, Z. Liu, J.A. Thompson, R. Agarwal, C. Agarwal, Fractionation of grape seed extract and identification of gallic acid as one of the major active constituents causing growth inhibition and apoptotic death of DU145 human prostate carcinoma cells, Carcinogenesis 27 (7) (2006) 1445–1453.

[23] (a) I. Kubo, K. Fujita, K. Nihei, Anti-*Salmonella* activity of alkyl gallates, J. Agric. Food. Chem. 50 (2002) 6692–6696;
(b) I. Kubo, P. Xiao, K. Fujita, Anti-MRSA activity of alkyl gallates, Bioorg. Med. Chem. Lett. 12 (2002) 113–116;
(c) I. Kubo, K. Fujita, K. Nihei, N. Masuoka, Non-antibiotic antibacterial activity of dodecyl gallate, Bioorg. Med. Chem. 11 (2003) 573–580;
(d) I. Kubo, K. Fujita, K. Nihei, A. Nihei, Antibacterial activity of alkyl gallates against *Bacillus subtilis*, J. Agric. Food. Chem. 52 (2004) 1072–1076;
(e) P.D. Stapleton, S. Shah, J.C. Anderson, Y. Hara, J.M. Hamilton-Miller, P.W. Taylor, Modulation of β-lactam resistance in *Staphylococcus aureus* by catechins and gallates, Int. J. Antimicrob. Agents 23 (5) (2004) 462–467.

[24] K. Fujita, I. Kubo, Antifungal activity of octyl gallate, Int. J. Food Microbiol. 79 (2002) 193–201.

[25] (a) C.J. Kane, J.H. Menna, C.-C. Sung, Y-Ch Yeh, Methyl Gallate, Methyl-3,4,5-trihydoxy-benzoate, is a potent and highly specific inhibitor of herpes simplex virus in vitro. II. Antiviral activity of methyl gallate and its derivatives, Biosci. Rep. 8 (1988) 95–102;
(b) J.M. Kratz, C.R. Andrighetti-Fröhner, P.C. Leal, R.J. Nunes, R.A. Yunes, E. Trybala, T. Bergström, C.R.M. Barardi, C.M.O. Simões, Evaluation of anti-HSV-2 activity of gallic acid and pentyl gallate, Biol. Pharma. Bull. 31 (5) (2008) 903–907;
(c) J.M. Kratz, C.R. Andrighetti-Fröhner, D.J. Kolling, P.C. Leal, C.C. Cirne-Santos, R.A. Yunes, R.J. Nunes, E. Trybala, T. Bergström, I.C.P.P. Frugulhetti, C.R.M. Barardi, C.M.O. Simões, Anti-HSV-1 and anti-HIV-1 activity of gallic acid and pentyl gallate, Mem. Inst. Oswaldo Cruz, Rio J. 103 (5) (2008) 437–442;
(d) L.A. Savi, P.C. Leal, T.O. Vieira, R. Rosso, R.J. Nunes, R.A. Yunes, T.B. Creczynski-Pasa, C.R. Barardi, C.M.O. Simões, Evaluation of anti-herpetic and antioxidant activities, and cytotoxic and genotoxic effects of synthetic alkyl-esters of gallic acid, Arzneim.-Forsch./Drug. Res. 55 (2005) 66–75;
(e) H. Yamasaki, M. Uozaki, Y. Katsuyama, H. Utsynomiya, T. Arakawa, M. Higuchi, T. Higuti, A.H. Koyama, Antiviral effect of octyl gallate against influenza and other RNA viruses, Int. J. Mol. Med. 19 (2007) 685–688.

[26] L. Nianguang, T. Yuping, D. Jinao CN101585769 (A)-2009-11-25.

[27] S. Benedikt, B. Coppens, J. Van Hemel, WO0130299-(A2)-2001-05-03.

[28] D. Lamoral-Theys, L. Pottier, F. Kerff, F. Dufrasne, F. Proutiere, N. Wauthoz, P. Neven, L. Ingrassia, P. Van Antwerpen, F. Lefranc, M.- Gelbcke, B. Pirotte, J.-L. Kraus, J. Nève, A. Kornienko, R. Kiss, J. Dubois, Simple di- and trivanillated exhibit cytostatic properties toward cancer cells resistant to pro-apoptotic stimuli, Bioorg. Med. Chem. 18 (11) (2010) 3823–3833.

[29] (a) K.J. Bell, A.N. Westra, R.J. Warr, J. Chartres, R. Ellis, Ch C. Tong, A.J. Blake, P.A. Tasker, M. Schröder, Outer-sphere coordination chemistry: selective extraction and transport of the $[PtCl_6]^{2-}$ anion, Angew. Chem. Int. Ed. 47 (2008) 1745–1748;
(b) F. Belin, P. Barthélémy, K. Ruiz, J.M. Lacombe, B. Pucci, Synthetic gallic acid derivatives as models for a comprehensive study of antioxidant activity, Helv. Chim. Acta 86 (2) (2003) 247–265;
(c) A.R. Bulls, C.G.F.E. Pippin, K.N.H. Raymond, Synthesis and characterization of a series of Vanadium-Tunichrome B1 analogues. Crystal structure of a tris(catecholamine) complex of vanadium, J. Am. Chem. Soc. 112 (1990) 2627–2632;
(d) R.J. Warr, A.N. Westra, K.J. Bell, J. Chartres, R. Ellis, C. Tong, T.G. Simmance, A. Gadzhieva, A.J. Blake, P.A. Tasker, M. Schröder, Selective extraction and transport of the $[PtCl_6]^{2-}$ anion through outer-sphere coordination chemistry, Chem. Eur. J. 15 (19) (2009) 4836–4850.

[30] N. Takashi, K. Kumiko, A. Akiko, M. Kenji, T. Koichiro, JP8143525 (A), 1996-06-04.

[31] (a) K.K. Bhargava, R.W. Grady, A. Cerami, New compounds: N^1, N^8-bis(2, 3-dihydroxybenzoyl)spermidine and analogs as potential iron-chelating drugs, J. Pharm. Sci. 69 (8) (1980) 986–989;
(b) E.J. Enemark, T.D.P. Stack, Spectral and structural characterization of two ferric coordination modes of a simple bis(catecholamide) ligand: metal-assisted self-assembly in a siderophore analog, Inorg. Chem. 35 (10) (1996) 2719–2720;
(c) L. Souleré, C. Viodé, J. Périé, P. Hoffmann, Selective inhibition of Fe- versus Cu/Zn-superoxide dismutases by 2,3-Dihydroxybenzoic acid derivatives, Chem. Pharm. Bull. 50 (5) (2002) 578–582;
(d) F.L. Weitl, K.N. Raymond, Specific sequestering agents for the actinides. 3. Polycatecholate ligands derived from 2,3-dihydroxy-5-sulfobenzoyl conjugates of diaza- and tetraazaalkanes, J. Am. Chem. Soc. 102 (1980) 2289–2293;
(e) K.J. Winstanley, D.K. Smith, Ortho-substituted catechol derivatives: the effect of intramolecular hydrogen-bonding pathways on chloride anion recognition, J.Org. Chem. 72 (8) (2007) 2803–2815.

[32] J. Paeshuyse, I. Vliegen, L. Coelmont, P. Leyssen, O. Tabarrini, P. Herdewijn, H. Mittendorfer, J. Easmon, V. Cecchetti, R. Bartenschlager, G. Puerstinger, J. Neyts, Comparative in vitro anti-hepatitis C virus activities of a selected series of polymerase, protease, and helicase inhibitors, Antimicrob. Agents Chemother. 52 (9) (2008) 3433–3437.

[33] M.P. Windisch, M. Frese, A. Kaul, M. Trippler, V. Lohmann, R. Bartenschlager, Dissecting the interferon-induced inhibition of hepatitis C virus replication by using a novel host cell line, J. Virol. 79 (2005) 13778–13793.

[34] L.J. Stuyver, T.R. McBrayer, P.M. Tharnish, A.E. Hassan, C.K. Chu, K.W. Pankiewicz, K.A. Watanabe, R.F. Schinazi, M.J. Otto, Dynamics of subgenomic hepatitis C virus replicon RNA levels in Huh-7 cells after exposure to nucleoside antimetabolites, J. Virol. 77 (19) (2003) 10689–10694.

[35] M.F. Bassendine, D.A. Sheridan, D.J. Felmlee, S.H. Bridge, G.L. Toms, R.D. Neely, HCV and the hepatic lipid pathway as a potential treatment target, J. Hepatol. 55 (6) (2011) 1428–1440.

[36] A.L. Rasmussen, D.L. Diamond, J.E. McDermott, X. Gao, T.O. Metz, M.M. Matzke, V.S. Carter, S.E. Belisle, M.J. Korth, K.M. Waters, R.D. Smith, M.G. Katze, Systems virology identifies a mitochondrial fatty acid oxidation enzyme, dodecenoyl coenzyme A delta isomerase, required for hepatitis C virus replication and likely pathogenesis, J. Virol. 85 (22) (2011) 11646–11654.

[37] M.T. Donato, M.J. Gómez-Lechón, Drug-induced liver steatosis and phospholipidosis: cell-based assays for early screening of drug candidates, Curr. Drug Metab. 13 (8) (2012) 1160–1173.

[38] S.E. Brevitt, E.W. Tan, Synthesis and *in vitro* evaluation of two progressive series of bifunctional polyhydroxybenzamide catechol-*O*-methyltransferase inhibitors, J. Med. Chem. 41 (1997) 2035–2039.

[39] (a) D.J. Brennan, A.P. Haag, J.E. White, C.N. Brown, High-barrier Poly(hydroxy amide ethers): effect of polymer structure on oxygen transmission rates, Macromolecules 31 (8) (1998) 2622–2630;
(b) G. Daidone, D. Raffa, B. Maggio, S. Plescia, M. Matera, A. Caruso, Synthesis and evaluation of the analgesic and antiinflammatory activities of N, N′ bis 2 hydroxybenzoyldiaminoalkanes, Il Farm. 45 (1990) 285–292.