

## Multivalent agents containing 1-substituted 2,3,4-trihydroxyphenyl moieties as novel synthetic polyphenols directed against HIV-1†‡

Cite this: *Org. Biomol. Chem.*, 2014, **12**, 5278

Aida Flores,<sup>a</sup> María José Camarasa,<sup>a</sup> María Jesús Pérez-Pérez,<sup>a</sup> Ana San-Félix,<sup>a</sup> Jan Balzarini<sup>b</sup> and Ernesto Quesada\*<sup>a</sup>

The synthesis and the assessment of the anti-HIV activity of a set of molecules inspired by the multivalent structures of some naturally-occurring polyphenols (tannins) are reported. Different multibranched scaffolds have been derived from pentaerythritol as the central core which distribute spatially synthetic polyphenolic subunits based on 1-substituted 2,3,4-trihydroxyphenyl moieties. A tetrapodal compound (**13b**) bearing four *N*-(2,3,4-trihydroxyphenyl)amide groups, exhibits remarkable selective activity against HIV-1 with EC<sub>50</sub> values in the micromolar scale, in the same range as those reported for the most representative anti-HIV tannins. Preliminary SAR studies emphasize the importance of the 1-substituted 2,3,4-trihydroxyphenyl moiety, the presence of an amide as the linker and the multivalent architecture of these molecules, since the anti-HIV activity increases with the number of polyphenolic moieties. The data support the interest in synthetic polyphenols and represent a promising starting point for further design and development of selective HIV-1 inhibitors.

Received 26th February 2014,  
Accepted 29th April 2014

DOI: 10.1039/c4ob00445k

www.rsc.org/obc

### Introduction

Acquired Immune Deficiency Syndrome (AIDS) remains a major global health concern due to its worldwide extension and the high mortality rate still associated with this disease.<sup>1</sup> Current antiretroviral therapy against Human Immunodeficiency Virus (HIV), the etiological agent of AIDS, consists of 26 clinically approved drugs,<sup>2</sup> which are administered in combination in the so-called *Highly Active Antiretroviral Therapy* (HAART).<sup>3</sup> This chemotherapeutic arsenal targets different critical stages of the infectious cycle of the virus and has converted AIDS from a lethal disease into a chronic, controlled condition.<sup>4</sup>

In spite of the positive effects of the HAART multidrug regime, the emergence of resistance to all drugs currently in clinical use as well as the appearance of side effects, particularly after long-term treatment, are major hurdles in the fight

against the virus.<sup>5,6</sup> Additionally, HAART does not eliminate the persistence of latent long-term reservoirs of HIV.<sup>7</sup> As a result, novel strategies and alternative therapeutic agents directed against HIV are still required.<sup>8</sup>

Polyphenols are a heterogeneous class of naturally-occurring compounds, usually secondary metabolites isolated from higher plants, which have been structurally characterised by the presence of polyhydroxy-aromatic moieties on their molecular frameworks.<sup>9</sup> Many polyphenols have been proposed as anti-HIV therapeutics, exerting different modes of action, ranging from virus entry, reverse transcription, integration and virus maturing.<sup>10</sup> Some of the most noticeable polyphenols described so far as agents against HIV include catechins,<sup>11</sup> theaflavins<sup>12</sup> (considered lately as inexpensive and safe microbicide candidates for the prevention of HIV sexual transmission<sup>13</sup>), or quercetin,<sup>14</sup> most of them found in various gallate forms.

Among them, the family of hydrolysable tannins has attracted particularly strong interest due to its remarkable activity against HIV. This heterogeneous group of polyphenols usually comprises a common architecture based on a multi-branched molecular core that spatially distributes several galloyl or galloyl-derived subunits, attached to the central scaffold through ester linkages.<sup>15</sup> Among these natural products must be noted some structurally simplified polyphenols such as 1,3,4,5-tetragalloylapiitol. This secondary plant metabolite contains an acyclic polyhydroxylated hydrocarbon core (D-apiitol) functionalised with four gallate moieties. This

<sup>a</sup>Instituto de Química Médica CSIC, Juan de la Cierva 3, 28006 Madrid, Spain.

E-mail: EQ1@iqm.csic.es; Fax: +34 91 564 48 53; Tel: +34 91 258 76 20

<sup>b</sup>Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

† Paper dedicated to Professor Richard J. K. Taylor on the occasion of his 65th birthday.

‡ Electronic supplementary information (ESI) available: Detailed experimental procedures for the synthesis of described compounds, as well as characterization data and copies of <sup>1</sup>H and <sup>13</sup>C NMR spectra of key intermediates and final compounds, are provided. See DOI: 10.1039/c4ob00445k



tannin exemplifies how a structurally simple polyol can act as an efficient distributing scaffold for polyphenolic subunits resulting in a molecule endowed with anti-HIV activity.<sup>16</sup>

The biological activities associated with polyphenols have been mainly attributed to the presence of differently functionalised polyhydroxy-aromatic moieties on their structures. In particular, galloyl subunits are structural motifs widely found in natural polyphenols. The presence of these groups constitutes an essential requisite for any bioactivity in most polyphenolic natural products.<sup>17</sup> Very noticeably, flavonols lacking galloyl moieties do not provide effective protection against HIV-1 infection and the number and position of free phenol (–OH) groups determine their biological properties.<sup>18</sup>

Galloyl residues provide polyphenols with efficient donor and acceptor-functionalities for hydrogen bonding, which give these compounds the potential to bind to proteins and nucleic acids.<sup>19,20</sup> Moreover, galloyl-containing polyphenols are notable anti-oxidants. A large body of evidence indicates that the oxidative environment facilitates viral replication<sup>21</sup> and that HIV infection is enhanced by free-radical damage.<sup>22</sup> In this sense, it has been suggested that gallate groups attenuate AIDS progression due to their anti-oxidant properties<sup>23</sup> which weaken the effects of the infection and the ability of the virus to infect new cells.<sup>24</sup>

As previously mentioned, a remarkable feature of the hydrolysable tannins is their multi-branched structure. Thus, several subunits of the polyhydroxy-aromatic moieties (*i.e.* gallic acid) are distributed profusely at the periphery of the scaffolds. This architecture provides these compounds with a dense outer array of repeated polyphenolic motifs (multivalency).<sup>25</sup> Actually, the multivalent concept of molecular design is currently starting to be successfully employed in anti-HIV drug discovery.<sup>26,27</sup> The combined above-mentioned factors (hydrogen bonding ability, anti-oxidative effect and multivalent architecture) allow the biological effect of polyphenols at different levels.

Albeit their intimate mechanisms of action have not been characterized in full detail, polyphenols have been proposed as useful candidates, either alone or in combination with conventional therapeutics, for the treatment and prevention of HIV-1 infection.<sup>10a</sup> However, naturally-occurring polyphenols are obtained as mixtures of compounds extracted from their natural sources. This is a significant drawback because in many cases antiviral activities are due to complex mixtures of substances (plant extracts) whose composition can only be estimated while further isolation of the active components from the extracts can turn out to be an elusive and difficult task. As a consequence, the development of chemical procedures able to allow access to pure, controlled samples of polyphenols is of great interest. Furthermore, naturally-occurring polyphenols can motivate and inspire researchers to look for novel synthetic compounds based on related architectures and/or new structurally-modified polyphenolic moieties. Surprisingly, and despite this interest, polyphenols have been only rarely explored as inspirational motifs of new anti-HIV agents. Moreover, synthetic polyphenols described to date are

analogues in which changes were focused on the nature of the scaffolds that distribute spatially the most ubiquitous polyphenolic moiety, the galloyl residue.<sup>28</sup> Thus, the promising antiviral profile of natural polyphenols and the lack of previous work on structurally-modified analogues of these compounds prompted us to initiate a program pursuing the discovery of multivalent compounds containing novel synthetic polyphenolic subunits.

In this sense, we recently described a tripodal receptor containing a triethylbenzene scaffold attached by amide linkers to 2,3,4-trihydroxybenzoyl groups (a galloyl isomer). It was stated that this moiety allowed the formation of an intramolecular hydrogen bond between the bridged-amide function and the *ortho*-phenolic substituent, thus rigidifying the structures compared to galloyl-containing compounds (3,4,5-trihydroxyphenyl isomer). This pre-organisation provided this compound with selective recognition properties for mannose-based polysaccharides, which opened attractive prospects for its potential in anti-infective strategies, including enveloped viruses such as HIV.<sup>29</sup>

Based on these precedents, we describe herein the synthesis and biological evaluation against HIV of a series of compounds inspired by the architecture of some hydrolysable tannins (such as 1,2,4,5-tetragalloylapiitol) in which the naturally-occurring galloyl group (3,4,5-trihydroxybenzoyl moiety) has been replaced by a synthetic 2,3,4-trihydroxyphenyl nucleus functionalised at position 1 with different linkers that allow their attachment to suitable scaffolds (Fig. 1).

As scaffolds, a series of simple mono-, bi-, tri- and tetrapodal molecules were used to explore the role of the number of polyphenolic subunits grafted onto a given backbone (multivalency) in the biological activity. Thus, pentaerythritol was proposed as a versatile molecule for construction of the four-branched core.<sup>30</sup> The gradual loss of branches of the pentaerythritol core allowed access to tripodal and bipodal scaffolds, while simple linear hydrocarbon chains of different lengths were used for the monopodal molecules. In addition, alkyl chains (2 or 3 methylene groups) have been introduced as spacers to separate the polyphenolic subunits from the central core in order to provide to the scaffolds both flexibility and elongated branches, avoiding steric hindrance. Amide bonds are ubiquitous in nature and chemically stable,<sup>31</sup> so they were the first choice for the linking functionalities, but esters, carbamates, ureas or triazoles (all good hydrogen bond donor and acceptor functionalities) were also used in order to evaluate the role of the linker on the biological activity. Thus far, we describe the synthesis and anti-HIV activity of this new family of compounds designed as synthetic polyphenols.

## Results and discussion

### Synthesis

We prepared a first series of compounds in which the selected 2,3,4-trihydroxyphenyl moiety was linked to a polyacid scaffold by amide linkages. With this purpose, 2,3,4-trihydroxy aniline (**8**)



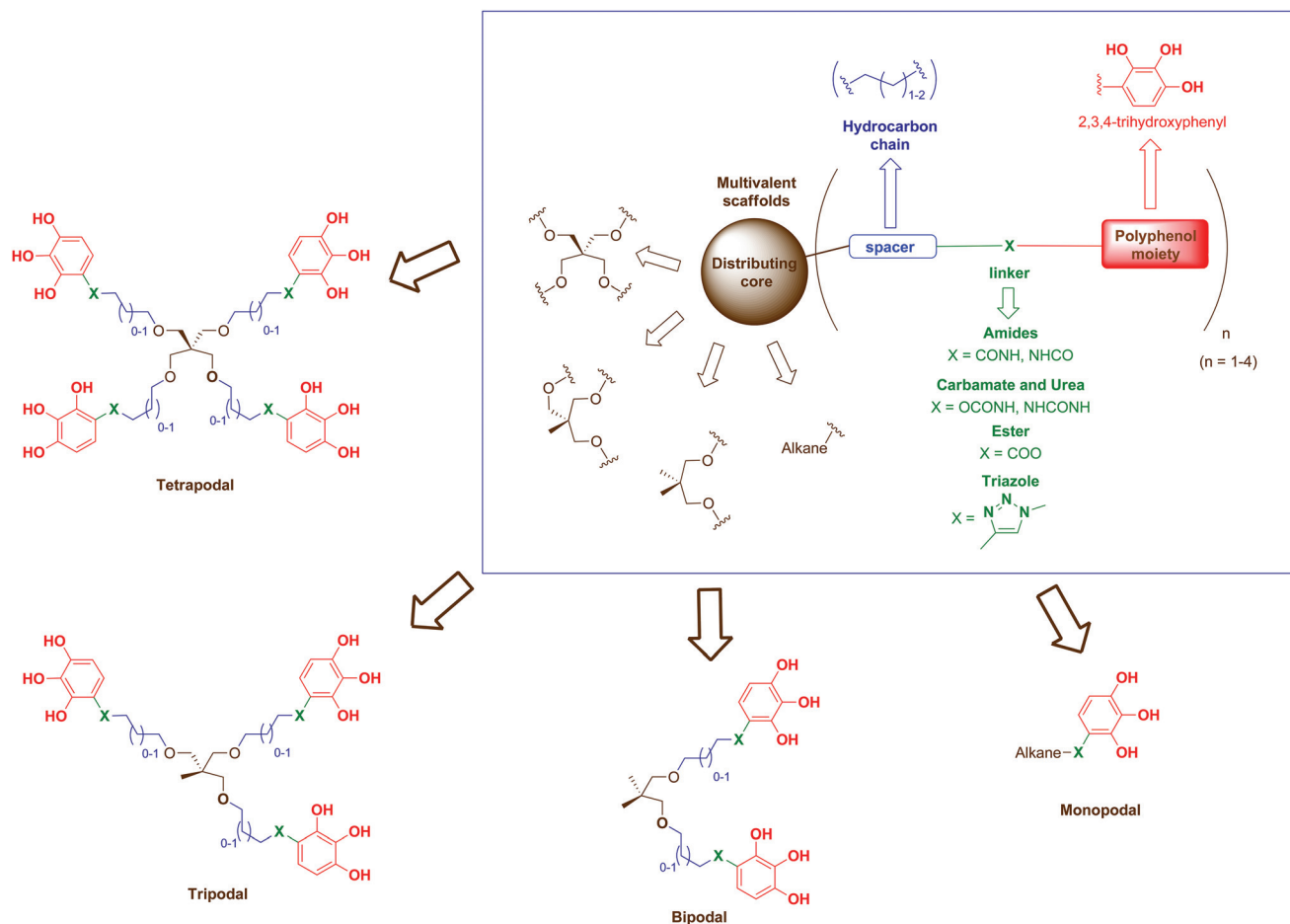
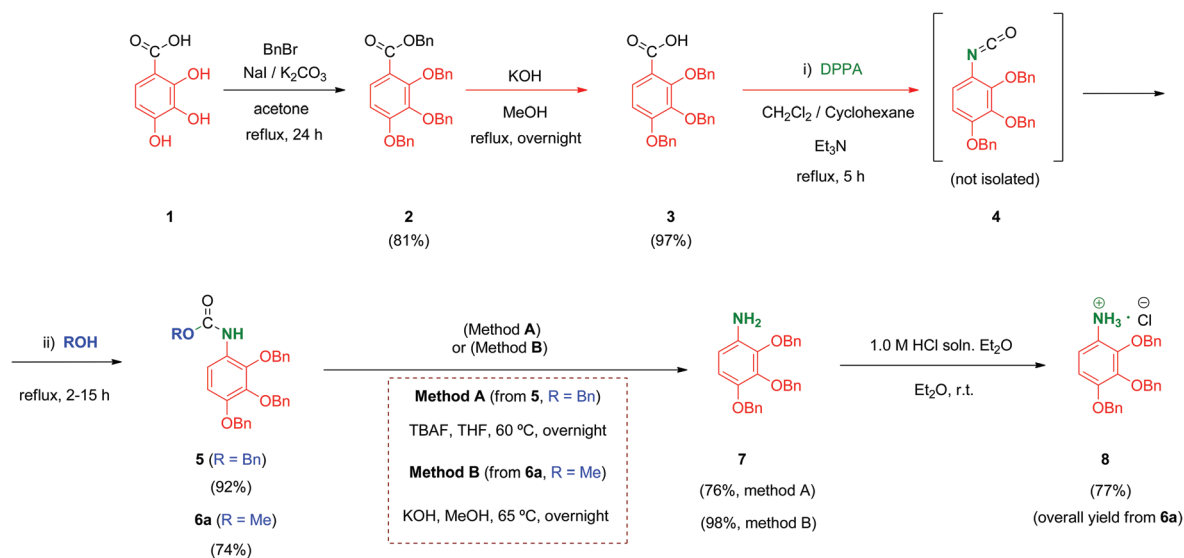


Fig. 1 Architecture of the proposed synthetic tannins.

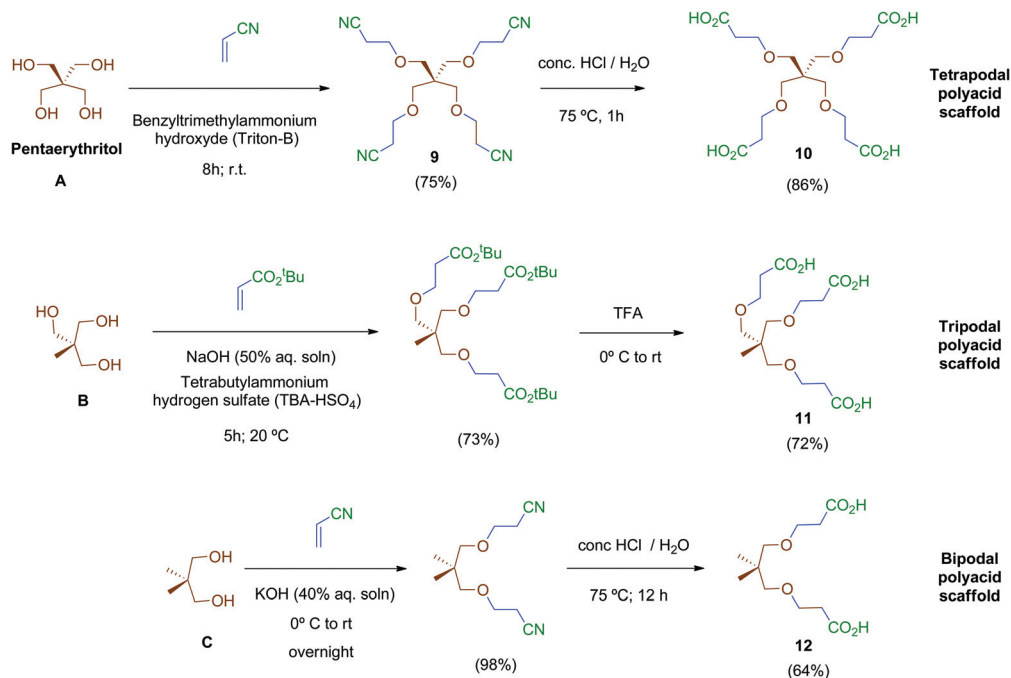


Scheme 1 Synthesis of amine 2,3,4-trihydroxybenzene nucleus 7 via Curtius rearrangement.

was prepared from the commercially available 2,3,4-trihydroxybenzoic acid (1) according to the pathway depicted in Scheme 1. Perbenzylation of the starting material 1 (acetone,

$K_2CO_3$ , heating),<sup>32</sup> followed by saponification of the benzyl ester, gave free acid 3<sup>33</sup> in high yield. Conversion of the benzoic acid 3 into its aniline analogue 7 was performed





**Scheme 2** Synthesis of multipodal polyacid scaffolds 10–12.

following a three-step synthetic sequence. The first transformation involved a Curtius reaction<sup>34</sup> using diphenylphosphoryl-azide (DPPA) as a mild azide-transfer reagent.<sup>35</sup> In this reaction, the corresponding acyl-azide derivative of **3** is formed and rearranged *in situ* to give the intermediate isocyanate **4**, which was not isolated from the reaction medium. A subsequent one-pot reaction of **4** with alcohols (BnOH, MeOH) produced the corresponding benzyl (**5**) and methyl (**6a**) carbamates respectively in high yield.<sup>36</sup> Removal of the Cbz (BnOCO) group in **5** was successfully achieved using an excess of tetrabutylammonium fluoride (TBAF) to afford the free aniline derivative **7** (Scheme 1, method A).<sup>37</sup> However, despite the satisfactory overall yield of the conversion, the large excess of TBAF that has to be used to drive the deprotection to completion and the toxicity of this reagent made it difficult to scale up this transformation. Alternatively, the methyl carbamate **6a** was efficiently deprotected by alkaline hydrolysis using KOH as reagent giving **7** in 98% yield. This procedure (Scheme 1, method B) performed on **6a** proved simpler from a practical point of view, higher yielding overall, easily scalable and inexpensive compared to the TBAF procedure (which is carried out specifically on the Cbz-protected aniline **5**).

Unfortunately, free aniline **7** proved to be unstable and decomposition was observed even when it was stored under controlled conditions (low temperature and inert atmosphere). To solve this limitation, **7** was converted immediately after isolation into its HCl salt **8**. Hence, hydrochloride **8** was stable and could be stored at room temperature for months without apparent degradation.

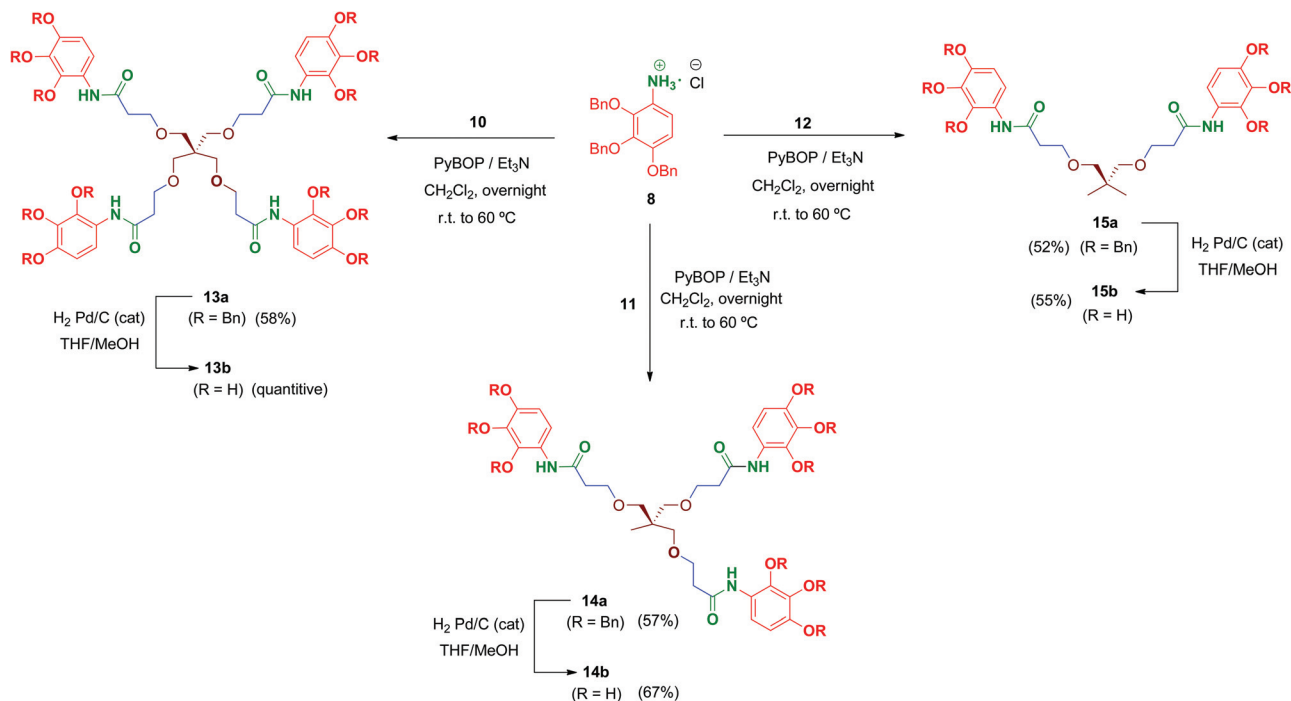
Next, scaffolds **10–12** with 4, 3 and 2 branched carboxylic acids, respectively, were synthesised following a two-step route

that involves firstly the conjugate addition of suitable polyols **A**, **B** and **C** to olefins with electron-withdrawing substituents followed by the conversion of the terminal functionalities (CO<sub>2</sub>tBu or CN) into the free acids carried out as specifically required (Scheme 2). Thus, tetraacid **10** was synthesised from the inexpensive, commercially available pentaerythritol (**A**), which reacts readily with acrylonitrile in aqueous media generating tetranitrile intermediate **9** (72% yield), which was finally hydrolysed providing the desired tetraacid **10** in 86% yield.<sup>38</sup> Tripodal scaffold **11** was synthesised from the commercially available triol **B** and *tert*-butyl acrylate in 73% yield.<sup>39</sup> *tert*-Butyl protecting groups were then straightforwardly removed by treatment with TFA, affording triacid **11** in 72% yield.<sup>40</sup> Finally, bidentate scaffold **12** was synthesised through a similar two-step sequence involving conjugate addition of commercially available diol **C** to acrylonitrile in 98% yield<sup>41</sup> and subsequent acid hydrolysis of nitrile groups to the corresponding free acids in 64% yield.

Multivalent benzyl-protected amides **13a–15a** were prepared by coupling of **8** and polyacid scaffolds **10–12** mediated by PyBOP/Et<sub>3</sub>N in dichloromethane as depicted in Scheme 3. Transformations proceeded at room temperature in 48 h, but gentle heating at 60 °C led the reaction to completion in shortened reaction times (overnight) and satisfactory yields.

Hydrogenolysis of all the benzyl-protected compounds **13a–15a** (H<sub>2</sub>, 3.1 bar, 25 °C, catalytic Pd/C) afforded the free-phenols **13b–15b** in good yields. The choice of benzyl as protecting group for the phenol functionalities proved successful as easy instrumental procedures and minimal work-up were required to achieve the fully deprotected compounds at high purity (≥95% HPLC).





Scheme 3 Synthesis of amide-linked tetra-, tri- and bipodal compounds 13–15.

In order to evaluate the influence on the activity of the position of the phenol groups on the aromatic ring, the 3,4,5-isomer derivative of 13b, namely compound 17b, was obtained (Scheme 4). This compound was synthesized similarly to 13b in 47% overall yield after two steps. The first transformation involves a PyBOP-mediated coupling of scaffold 10 and the corresponding 3,4,5-benzyloxy aniline 16 followed by hydrogenolysis of the benzyl groups in 17a to afford 17b. Additionally, with the aim of analysing the role of the free amide proton on the activity, the *N*-methyl derivative 18b was prepared in 41% overall yield by reaction of 13a with methyl iodide followed by removal of the benzyl groups in 18a by hydrogenation.

Next, to complete the amide series of compounds with monopodal structures and to properly evaluate the effect of branching on the scaffolds, compounds 19b–21b were prepared. Thus, 8 was straightforwardly functionalised with acetic anhydride in pyridine to afford acetate 19a. Ethyl (20a; short-chained derivative) and palmitoyl (21a; long-chained derivative) monopodal amides were synthesized by reaction of 8 with the corresponding acyl chlorides in order to additionally evaluate the role of the amphiphilic character of these molecules. Finally, hydrogenolysis of the benzyl groups of 19a–21a afforded the monopodal free-phenols 19b–21b in satisfactory yields (Scheme 5).

Preliminary biological evaluation of compounds 13b–21b indicated that the tetrapodal compound 13b showed promising anti-HIV-1 activity (see biological evaluation section). We then considered preparing the tetrapodal amide 23b in which the linker is reversed (NHCO instead of CONH), compared to 13b, in order to evaluate the influence of this structural parameter on the activity (Scheme 6). Thus, coupling of the suit-

able tetraamine 22 (prepared as will be discussed later) with the corresponding acid 3 afforded 23a, whose further deprotection yielded 23b in 69% yield.

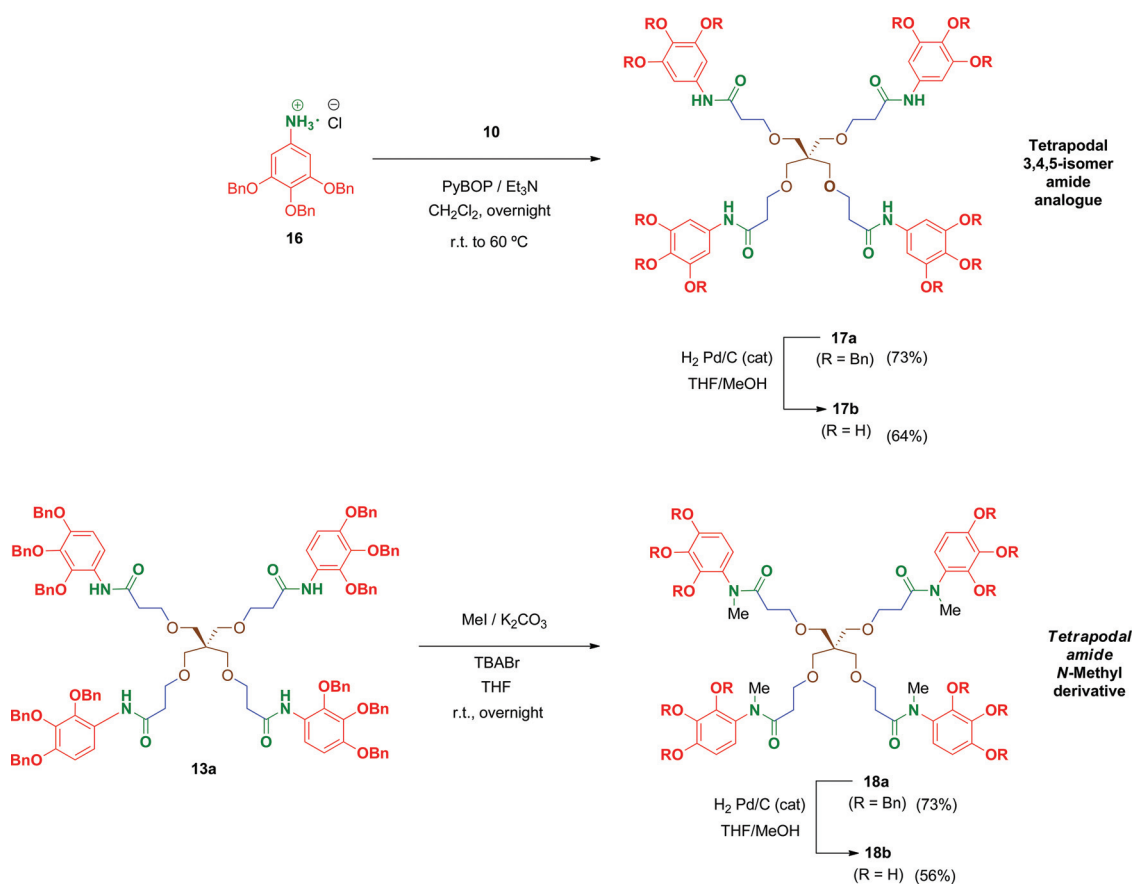
We were encouraged to extend the study to new molecules with modified linkers between the scaffold and the 2,3,4-trihydroxyphenyl moieties. Thus, isocyanate 4 emerged as a key intermediate that allowed its direct conversion into carbamate (OCONH) and urea-linked (NHCONH) derivatives, obtained straightforwardly by reaction of 4 with alcohols and amine-containing cores, respectively (Schemes 9 and 10). For the synthesis of these compounds, scaffolds 22 and 24–26 were prepared as shown in Schemes 7 and 8.

Treatment of tetraacid 10 with HCl gas in methanol<sup>42</sup> followed by reduction of the *in situ*-generated methyl ester intermediate with LiAlH<sub>4</sub> afforded tetraol 24. This sequential method proved more reliable than the direct reduction of the tetraacid 10 with borane–THF complex as described.<sup>43</sup> Tri- and bipodal alcohols 25<sup>44</sup> and 26<sup>45</sup> were obtained similarly by reduction of their corresponding methyl ester intermediates D and E (Scheme 7).

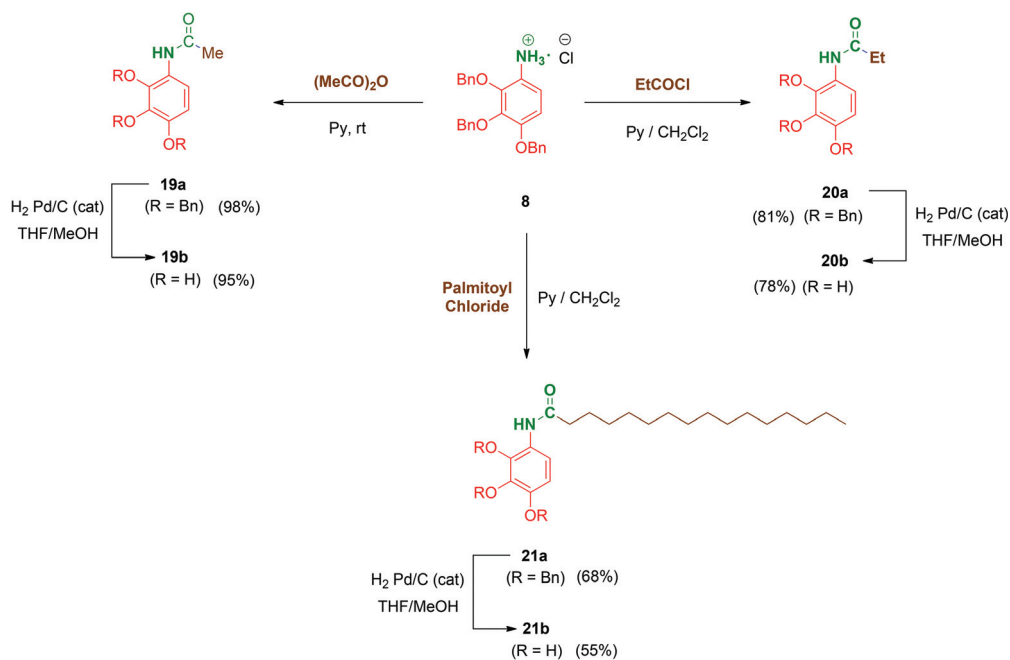
Conversion of the polyol 24 into the tetraamine 22 (Scheme 8) was performed firstly *via* tetraazide intermediate 27, which was obtained by a tandem procedure involving a Mitsunobu activation of the primary alcohols of 24 (DIAD, PPh<sub>3</sub>) followed by an *in situ* reaction with diphenylphosphorylazide (DPPA), acting as an azide-transfer reagent. This transformation allows a simple and straightforward synthesis of tetraazide 27 alternatively to the described procedure,<sup>46</sup> which involves previous activation of the alcohols as mesylates, isolation and subsequent conversion into their azide derivatives by reaction with sodium azide. Finally, 27 was reduced to the





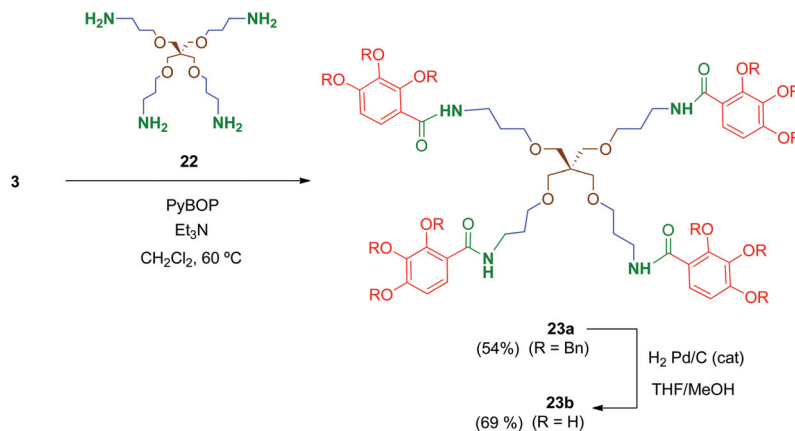
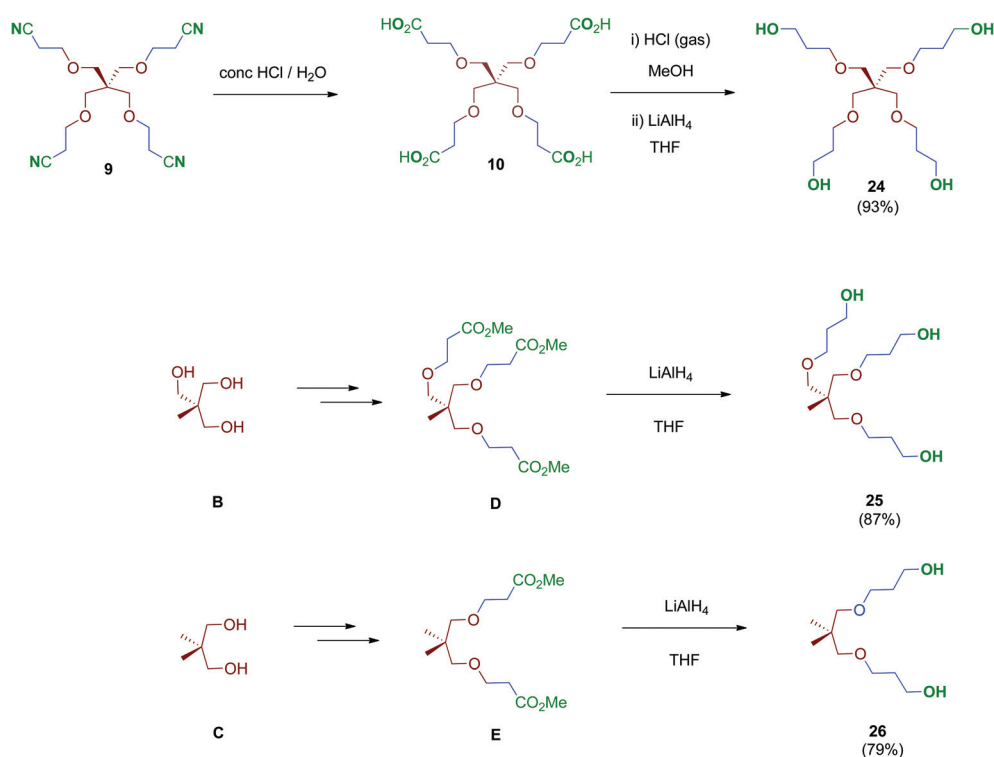


Scheme 4 Synthesis of amide-linked tetrapodal compounds 17–18.



Scheme 5 Synthesis of monopodal amide-linked compounds 19–21.



Scheme 6 Synthesis of tetrapodal amide **23b**.Scheme 7 Synthesis of tetrapodal, tripodal and bipodal polyol scaffolds **24–26**.

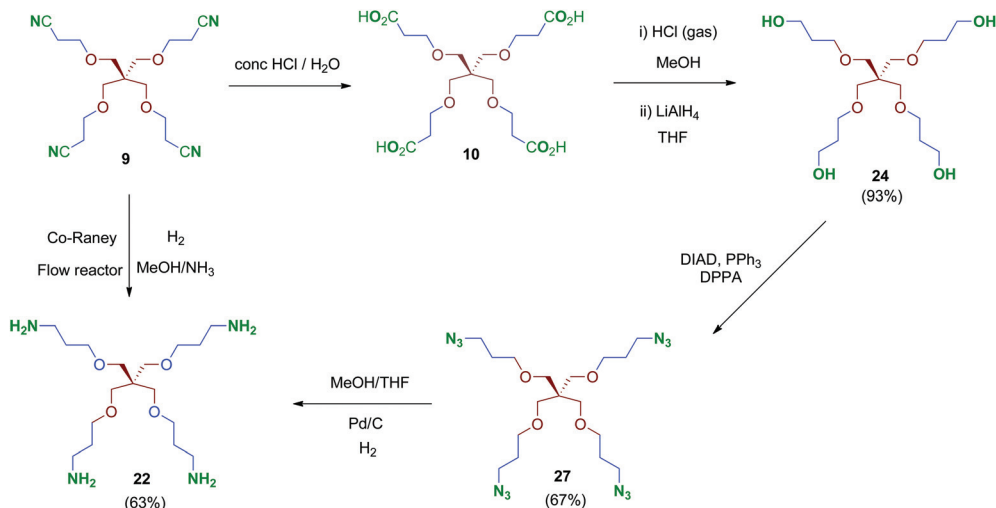
tetraamine **22** in 63% yield by catalytic hydrogenation under standard conditions. Alternatively, compound **22** could be obtained directly from the tetranitrile **9** by hydrogenation at high pressure (70 bar, 70 °C; Scheme 8). This reaction was performed in a continuous-flow reactor using immobilized Co-RANEY® as the catalyst, providing the tetraamine in a single step and quantitative yield. This was finally the method of choice to reach the tetravalent amine-containing scaffold **22**.

Reaction of polyols **24–26** with **4**, generated *in situ* by the treatment of **3** with DPPA as previously mentioned, afforded carbamates **28a–30a** in reasonable yields (Scheme 9). Debenzylation by hydrogenation of **28a–30a** proceeded satisfactorily affording free polyphenols **28b–30b**. Carbamate-linked com-

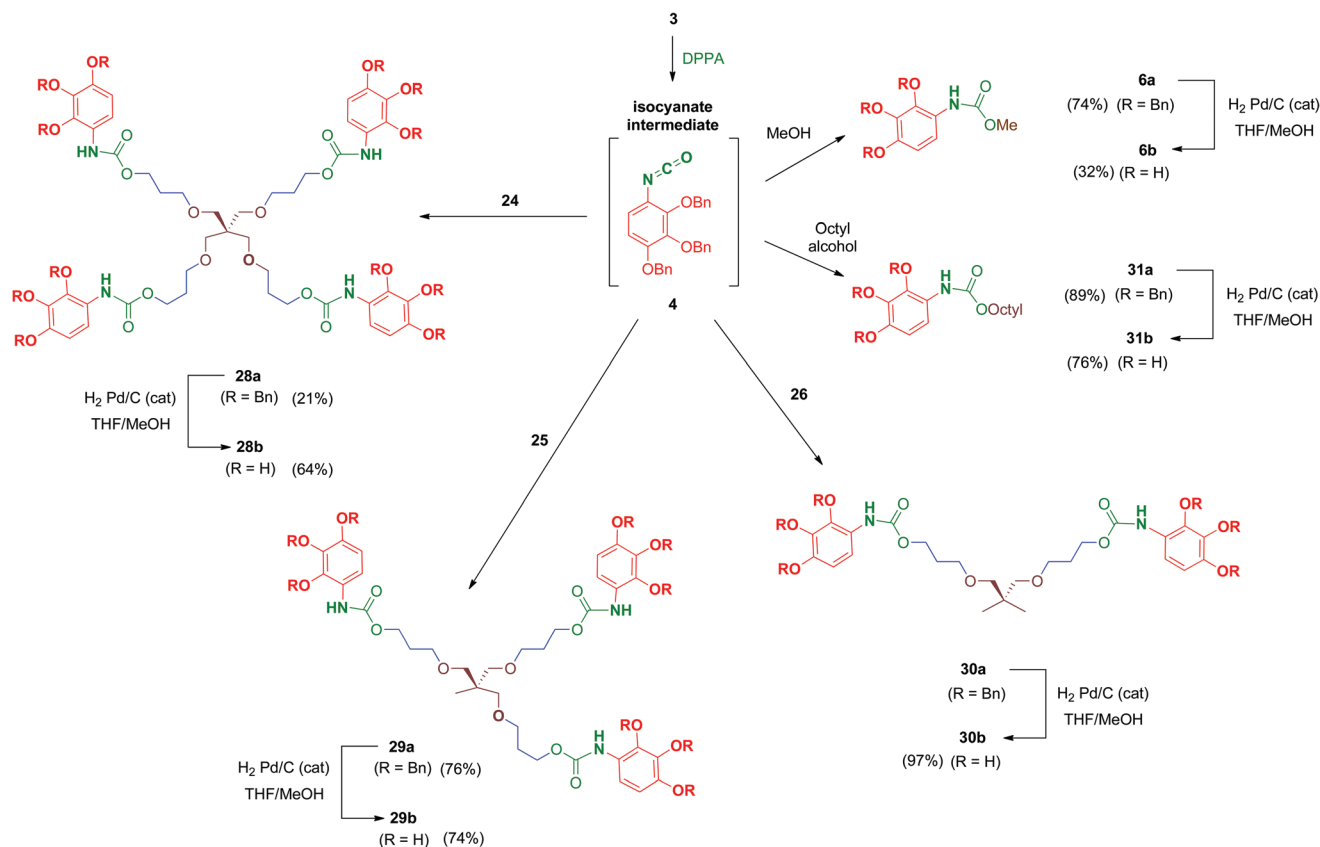
pounds were completed with the monodentate methyl derivative **6b** (the synthesis of this benzyl-protected derivative was previously described) and the octyl derivative **31b** in order to evaluate the effect of the amphiphilic nature of the carbamate.

Regarding urea-linked derivatives, tetradentate amine **22** (Scheme 10) reacted very poorly with isocyanate **4**. Thus, only by forcing reaction conditions under microwave irradiation (100 °C for 90 min) was it possible to isolate tetrapodal urea **32a**, albeit with a poor 17% yield. Additionally, reaction of **4** with propylamine afforded monodentate compound **33a** in 52% yield. Monodentate urea **33b** containing free phenol groups was successfully isolated in 99% yield after hydrogenation. However, tetradentate urea **32b** was not obtained pure





Scheme 8 Synthesis of tetrapodal polyamine (22) and polyazide (27) scaffolds.



Scheme 9 Synthesis of carbamate-linked series from key isocyanate 4.

after removal of the benzyl protecting groups in **32a**. In this case, the material isolated after hydrogenolysis contained several minor by-products. Attempts to purify this crude product (reverse-phase chromatography, recrystallization, exchange resins, *etc.*) failed to increase purity of this tetradentate urea. Thus, exceptionally, compound **32b** was evaluated

biologically against HIV with a purity of 82% (as determined by HPLC).

As will be seen in the biological section, the data obtained from the evaluation against HIV of the amide (**13b–15b**, **17b–21b**) and carbamate (**6b**, **28b–31b**) series clearly indicated that the more 2,3,4-trihydroxyphenyl moieties on the periphery







**Scheme 10** Synthesis of tetrapodal and monopodal urea-linked compounds from isocyanate 4.

of the molecules, the better the anti-HIV activity. Thus, our next series of structural modifications were performed based on these findings and we decided to focus only on the tetrapodal compounds, while the monopodal derivatives were obtained for comparative purposes.

Next, compounds with an ester as the linker were prepared (Scheme 11). In this case, derivative 36 was the key intermediate. This compound was obtained in a three-step sequence by reduction of 2 with lithium aluminium hydride under standard conditions (LiAlH<sub>4</sub>, THF) to afford benzyl alcohol 34 in 80% yield.<sup>47</sup> Subsequent conversion of this alcohol into its aldehyde derivative 35 was initially performed by using pyridinium chlorochromate (PCC) as the oxidizing agent (Scheme 11, method A) as described in the literature for the isomeric 3,4,5-trimethoxy analogue of 34.<sup>48</sup> However, this transformation afforded, after chromatography, only moderate yields (45%) of the desired aldehyde 35. Alternatively, a MnO<sub>2</sub>-mediated oxidation (Scheme 11, method B) was assayed giving aldehyde 35 in better yield (69%) and avoiding tedious chromatographic purifications. Finally, a Dakin-type reaction (H<sub>2</sub>O<sub>2</sub>-HCO<sub>2</sub>H) converted the aromatic aldehyde 35 into its phenol derivative 36 as described.<sup>49</sup>

Coupling of compound 36 with the tetra-acid scaffold 10 was achieved readily by Steglich reaction mediated by DCC and a catalytic amount of DMAP to give tetradentate ester 37a in 62% yield. A simple monodentate ester 38a was also obtained for comparative purposes by treatment of 36 with

acetic anhydride in pyridine. Finally, removal of the benzyl ethers in 37a and 38a was carried out by hydrogenolysis using the standard protocol. Deprotected compound 38b undergoes a phenomenon of intramolecular acyl migration between vicinal phenols, producing spontaneously in the hydrogenation medium a (2 : 1) mixture of 1-acetyl/2-acetyl isomers 38b/38c as determined by <sup>1</sup>H-NMR. Tetraester 37b did not exhibit this spontaneous trend of transesterification between the phenol groups, although all compounds 37b and 38b/38c proved labile in solution, probably due to partial hydrolysis of the ester moieties.

Finally, in order to explore new structural modifications on the linker, we considered it of interest to replace the amide bridge of the most active tetrapodal compound 13b by a non-hydrolysable surrogate. The triazole group has been proposed as a non-classical bioisostere of the amide moiety retaining the main parameters of the original group (electronic properties, lipophilicity, size and geometry) while introducing structural diversity. Additionally, triazole groups have the advantages of a remarkable chemical stability and easy generation (click chemistry).<sup>50</sup> Thus, two non-hydrolysable triazole-linked tetrapodal agents 40b and 43b were synthesized as shown in Scheme 12. Two different azide-alkyne pairs were used. For the synthesis of 40b, acetylene 39 was required. Compound 39 was obtained from the benzyl alcohol 34 through a sequential one-pot oxidation-propargylation protocol.<sup>51</sup> Thus, 34 was oxidised by activated MnO<sub>2</sub> (THF, reflux) with no



Scheme 11 Synthesis of monopodal and tetrapodal esters **37b** and **38b/38c**.

Scheme 12 Synthesis of tetrapodal triazole-linked polyphenols.

isolation of intermediate aldehyde **35**, which was transformed *in situ* into the corresponding acetylene **39** by treatment with dimethyl-(1-diazo-2-oxopropyl)phosphonate (Bestmann–Ohira

reagent) in the presence of K<sub>2</sub>CO<sub>3</sub> at room temperature.<sup>52</sup> Subsequently, Huisgen reaction (Cu(I)-catalysed [1,3]-dipolar cycloaddition)<sup>53</sup> between **39** and the previously mentioned



tetraazide **27** in the presence of  $\text{CuSO}_4$  and sodium ascorbate afforded tetra-triazole **40a** in good yield (81%).

On the other hand, tetrapodal derivative **43a** was obtained by coupling of aryl-azide **41** and its acetylenic pentaerythritol counterpart **42**. Compound **41** was obtained from the hydrochloride **8**, which was converted into its diazo derivative (not isolated) by treatment with sodium nitrite and subsequently substituted *in situ* by treatment with sodium azide to afford **41**, although in low yield (23%; non-optimised procedure). Huisgen coupling of **41** with **42** (synthesized by O-propargylation of pentaerythritol<sup>54</sup>) provided **43a** in satisfactory yield (60%). Finally, removal of benzyl protecting groups in both **40a** and **43a** was carried out under standard conditions affording deprotected polyphenols **40b** and **43b** in 60% and 85% yield, respectively.

### Biological assays. Antiviral activity

The new synthetic polyphenols were evaluated for their inhibitory activity against HIV-1(III<sub>B</sub>) and HIV-2 (ROD) in a cell-based assay ( $\text{CD}_4^+$  T-lymphocyte cell culture) where virus infected cells were incubated in the presence of the selected compounds.

It must be noted firstly that none of the benzyl-protected polyphenols exhibited activity against HIV at subtoxic concentrations (data not shown), which clearly indicates that free phenol functions are crucial for any antiviral activity in this family of compounds. The results of the biological evaluation of the deprotected compounds against HIV are summarized in Table 1.

Whereas some of the described compounds inhibited HIV-1 replication in the lower micromolar concentration range, none of these derivatives proved active against HIV-2 at sub-toxic concentrations (Table 1). Therefore, the active compounds should be considered as specific inhibitors of HIV-1 replication in  $\text{CD}_4^+$  T-lymphocyte cells.

With respect to the architecture of the tested compounds, it was shown that the number of polyhydroxyphenyl moieties is important for the activity. Thus, monopodal (**6b**, **19b–21b**, **31b**, **33b** and **38b,c**) and bipodal (**15b** and **30b**) compounds were all inactive against HIV replication at subtoxic concentrations. In the monopodal series of amides (**19b–21b**) and carbamates (**6b** and **31b**), the length of the hydrocarbon side chain (Me, Et, octyl and palmitoyl) linked to the phenolic moiety did not affect their antiviral behaviour, indicating that the hydrophobic character of the molecule has no significant effect on activity. It should be noted additionally that compound **21b**, bearing a long palmitoyl hydrocarbon chain, has a noticeably amphiphilic nature, which precluded its biological testing at a higher concentration of 2  $\mu\text{M}$  due to its limited aqueous solubility.

Among the tripodal derivatives, only compound **14b** exhibited a pronounced anti-HIV-1 activity ( $\text{EC}_{50} = 4.2 \mu\text{M}$ ) although its activity is 5-fold lower than its toxicity threshold (selectivity index  $\sim 6$ ).

Regarding the tetrapodal series, compounds **40b** and **43b**, with non-hydrolysable triazole linkers, and **37b** and **32b**, connected to the scaffold with ester and urea linkers respectively, were all inactive. In contrast, the tetrapodal amide-linked

**Table 1** Anti HIV-1(III<sub>B</sub>) and anti-HIV-2(ROD) activity and cytostatic properties of the test compounds in human  $\text{CD}_4^+$  T-lymphocyte (CEM) cell cultures

Linker	Multiplicity	Compound	$\text{EC}_{50}^a$ ( $\mu\text{M}$ )		$\text{CC}_{50}^b$ ( $\mu\text{M}$ )
			HIV-1	HIV-2	
Carbamate	Monopodal	<b>6b</b>	>10	>10	171 $\pm$ 85
Amide	Tetrapodal	<b>13b</b>	1.2 $\pm$ 0.6	$\geq 10$	25 $\pm$ 11
Amide	Tripodal	<b>14b</b>	4.2 $\pm$ 0.85	>10	24 $\pm$ 8.5
Amide	Bipodal	<b>15b</b>	>10	>10	60 $\pm$ 5.4
Amide (3,4,5-isomer)	Tetrapodal	<b>17b</b>	5.6 $\pm$ 1.6	>10	21 $\pm$ 5.7
Amide ( <i>N</i> -Me derivative)	Tetrapodal	<b>18b</b>	>10	>10	63 $\pm$ 37
Amide	Monopodal	<b>19b</b>	>50	>50	112 $\pm$ 0.0
Amide	Monopodal	<b>20b</b>	>50	>50	108 $\pm$ 2.1
Amide	Monopodal	<b>21b</b>	>2 <sup>c</sup>	>2 <sup>c</sup>	>2 <sup>c</sup>
Amide (NHCOAr)	Tetrapodal	<b>23b</b>	10 $\pm$ 0.0	>10	21 $\pm$ 2.8
Carbamate	Tetrapodal	<b>28b</b>	8.4 $\pm$ 2.3	>10	20 $\pm$ 4.2
Carbamate	Tripodal	<b>29b</b>	>10	>10	24 $\pm$ 4.9
Carbamate	Bipodal	<b>30b</b>	>50	>50	86 $\pm$ 17
Carbamate	Monopodal	<b>31b</b>	>10	>10	124 $\pm$ 11
Urea	Tetrapodal	<b>32b<sup>d</sup></b>	>10	>10	22 $\pm$ 0.0
Urea	Monopodal	<b>33b</b>	>50	>50	>250
Ester	Tetrapodal	<b>37b</b>	>50	>50	102 $\pm$ 21
Ester	Monopodal	<b>38b/38c</b>	>50	>50	>250
Triazole	Tetrapodal	<b>40b</b>	>10	>10	27 $\pm$ 2.8
Triazole	Tetrapodal	<b>43b</b>	>10	>10	48 $\pm$ 30

<sup>a</sup> 50% effective concentration or compound concentration required to protect 50% of the cells against the cytopathic effect of the virus. <sup>b</sup> 50% cytostatic concentration or compound concentration required to inhibit CEM cell proliferation by 50%. All data are mean values (standard deviation for at least three independent experiments). <sup>c</sup> Compound precipitation was detected at higher concentrations. <sup>d</sup> Product evaluated with 82% purity.



derivative **13b** was endowed with the highest antiviral activity against HIV-1 ( $EC_{50} = 1.2 \mu\text{M}$ ), a concentration markedly below the toxicity threshold, resulting in a selectivity index of  $\sim 20$ . Very noticeably, reversion in the sequence of the amide linker (NH-CO in **17b** instead of CO-NH in **13b**) caused a 4- to 5-fold loss of antiviral activity (**17b**,  $EC_{50} = 5.6 \mu\text{M}$ ) keeping similar levels of cytostatic activity ( $CC_{50} = 21 \mu\text{M}$ ). Also, the tetrapodal derivative **28b** attached to the scaffold through a carbamate group (NH-CO-O) was 7-fold less active than **13b** ( $EC_{50} = 8.4 \mu\text{M}$ ), linked by an amide (NHCO) moiety, but again, kept a similar cytostatic potential as **13b** and **17b**. Overall, it can be concluded that the best architecture is the tetrapodal configuration and that, despite a common tetrapodal scaffold, the linker group clearly affects the activity.

The amide series (compounds **13b–21b**) can help to establish interesting structure–activity relationships. On the one hand, the distribution of the free phenolic OH groups around the aromatic ring is important for the biological activity. Thus, compound **17b**, bearing polyphenolic subunits with a galloyl-type distribution (3,4,5-trihydroxyphenyl moieties), was 7-fold less active ( $EC_{50} = 5.6 \mu\text{M}$ ) than compound **13b**, substituted by the 2,3,4-trihydroxyphenyl isomer, showing that this moiety is superior for this series of compounds. Moreover, compound **18b**, in which amide NH groups have been eliminated by the introduction of methyl groups, was inactive compared to **13b**. This indicates the importance of the free NH groups probably to keep the capacity to form H-bridges with the molecular target. Finally, compared to its less branched tripodal (**14b**), bipodal (**15b**) and monopodal (**19b–21b**) analogs, the antiviral profile of **13b** indicates that at least four 2,3,4-trihydroxyphenyl moieties located at the periphery of the molecule are required for antiviral activity. These data support the importance of a multi-branched architecture in the design of these families of compounds. Moreover, it is interesting to notice that the cytostatic activity of the antivirally active test compounds were quite comparable, which opens promising future prospects of further improvement of the selectivity index by optimizing (increasing) the antiviral potency.

## Conclusions

Herein we have described the synthesis and anti-HIV properties of a series of synthetic polyphenols containing a central pentaerythritol-based core functionalised with 2,3,4-trihydroxyphenyl subunits. Amide, carbamate, urea, ester and triazole groups have been used as linkers to attach the polyphenolic moieties to the scaffolds. The design of these compounds has been inspired by the multivalent architecture of some representative naturally-occurring tannins. The effect of the number of polyphenolic moieties has been studied by the synthesis of a tetra-, tri- and bipodal series of compounds while monopodal derivatives were obtained for comparative purposes. Multibranch architecture seems to play a crucial role in the design of these compounds, since the increase in the number of peripheral polyphenolic subunits is associated with an increased activity.

The tetrapodal amide-linked compound **13b** is the most active member of this series, exhibiting low micromolar inhibitory activity against HIV-1, in the same range or even improved compared to those values that have been reported for related naturally-occurring polyphenols.<sup>10a</sup> The 2,3,4-trihydroxyphenyl moiety present in these compounds proved superior to the naturally-occurring gallate group (3,4,5-trihydroxybenzoyl moiety). It is noteworthy that any structural changes performed on the linker of the prototype amide-bridged compound **13b** led to a decrease in antiviral activity. The specific molecular target of prototype compound **13b** as well as its exact mechanism of action are both still under investigation.

Furthermore, it must be noted that naturally-occurring polyphenols are extracts from natural sources whose composition is variable, while the synthesis of the compounds described herein has been carried out through a set of simple and reliable transformations that allowed the isolation of pure, controlled samples of the substances ready for biological evaluation.

For all the above reasons, this contribution can be considered as an encouraging starting point for further development of synthetic polyphenols as selective HIV-1 inhibitors.

## Experimental

### Chemical synthesis

**General methods.** Reactions were carried out with magnetic stirring in round-bottomed flasks unless otherwise noted. Air or moisture-sensitive reactions were conducted in oven-dried glassware under a positive pressure of dry argon. Microwave-mediated reactions were performed in a Biotage Initiator™ 2.0 reactor. Debenzylation of benzyl-protected phenols was performed in a Parr-hydrogenator working at 3.1 bar and thermostated at 25 °C. High-pressure hydrogenation of tetranitrile **9** was performed on an H-Cube® Continuous-flow Hydrogenation reactor using Co-RANEY® as catalyst immobilized in a CatCart®-Catalyst Cartridge System (both from Thales Nano). All solvents were pre-dried, freshly distilled prior to use as specifically described.<sup>55</sup> Analytical thin layer chromatography (TLC) was performed on pre-coated aluminum silica gel plates 60 ( $F_{254}$ , 0.25 mm). Products were visualized from the TLC under UV lamps (254 or 260 nm) or by heating after treatment with developer solution (cerium ammonium molybdate (CAM), phosphomolybdic acid (PMA) or vanillin). Separations were performed on silica gel by preparative flash column chromatography<sup>56</sup> or by Centrifugal Circular Thin-Layer Chromatography (CCTLC). Large scale purifications were conducted on an automated flash purification system apparatus (Biotage Isolera One). Oils or waxy solids were converted into foams by repeated freeze-drying (lyophilization) from acetonitrile or DMSO and traces of water for better handling of the products.

Mass spectra (low and high-resolution) were registered with a quadrupole mass spectrometer Hewlett-Packard 1100 equipped with an electrospray source (ES) in negative or positive mode of detection. NMR spectra were obtained on Varian





INOVA-300, -400, UNITY-500, MERCURY-400 or Bruker AVANCE-300 NMR spectrometers using  $\text{CDCl}_3$  or methanol- $d_4$  as solvents.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra are reported in parts per million (ppm) referred to protonated residual peaks or specific signals due to deuterated solvents as internal references in  $\text{CDCl}_3$  or  $\text{CD}_3\text{OD}$ . The following abbreviations are used to describe peaks: s (singlet), d (doublet), t (triplet), qt (quartet), qn (quintet), m (multiplet) and br (broad).

HPLC analyses were carried out using either a Waters Alliance HPLC/MS system (quoted as **HPLC/MS**) or on an Agilent 1120 apparatus (quoted as **HPLC**) eluting with acetonitrile-water. All retention times are quoted in minutes. Elemental analyses were performed with a Carlo Erba CHN-1108 or a Heraeus CHN-O-RAPID microanalyzers. Melting points were measured on a Mettler Toledo model MP70 melting point apparatus and are uncorrected. Lyophilised compounds melt at a wide range of temperatures.

A representative set of compounds are described in this Experimental section. A detailed full description of all the analytical methods, experimental procedures, spectroscopic characterization and selected copies of  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra of intermediates and final compounds are provided separately in the ESI $^\ddagger$  available.

#### Preparation of 2,3,4-tris(benzyloxy)aniline (7) from carbamate precursors

**Method A. Reaction with TBAF. Specific method for benzyl-carbamate deprotection.** Cbz-protected aniline **5** (1.1 g, 2 mmol) was dissolved in dry, freshly distilled THF (5 mL) under argon atmosphere at room temperature. A solution of TBAF in THF (1 M solution, 30 mL, 15 equiv.) was added and the reaction mixture was heated at 60 °C overnight. TLC monitoring indicated complete deprotection of carbamate. Volatiles were removed under vacuum and the residue embedded in a pad of celite and directly purified by column chromatography on  $\text{SiO}_2$  (hexane-EtOAc (2 : 1) v/v as eluent). Free amine **7** was isolated as a waxy solid, converted into a white foam after repeated freeze-drying from  $\text{CH}_3\text{CN-H}_2\text{O}$  (627 mg, 1.5 mmol, 76%). TLC ( $R_f$  = 0.32; hexane-EtOAc (3 : 2) v/v; CAM);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 7.45–7.3 (m, 15 H), 6.6 (d,  $J$  = 8.8 Hz, 1H), 6.49 (d,  $J$  = 8.8 Hz, 1H), 5.1 (s, 2H), 5.07 (s, 2H), 5.04 (s, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 145.5, 143.4, 140.8, 137.9, 137.8, 137.7, 135.5, 128.6, 128.5, 128.4, 128.2, 128.0, 127.9, 127.8, 111.7, 110.1, 75.7, 75.2, 72.5;  $m/z$  (ESI $^+$ ) 434 ([ $\text{M} + \text{Na}$ ] $^+$ , 26%), 412 ([ $\text{M} + \text{H}$ ] $^+$ , 100%); HPLC/MS:  $t_R$  = 2.55 min. polar gradient  $\text{CH}_3\text{CN-H}_2\text{O}$  from (70 : 30) up to (100 : 0) in 5 min (98% purity).

**Method B. Alkaline hydrolysis of carbamates. General procedure.** A 40% solution of KOH in methanol (5 mL  $\text{mmol}^{-1}$ ) was added to the methyl carbamate **6a** (1 g, 1.83 mmol). The slurry was stirred under reflux overnight (monitored by TLC). Then, it was cooled to room temperature and the solvent was halved, diluted with  $\text{H}_2\text{O}$  (10 mL  $\text{mmol}^{-1}$ ) and extracted with EtOAc (3  $\times$  10 mL  $\text{mmol}^{-1}$ ). Combined organic layers were dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under vacuum to yield the desired free amine **7**, which was triturated

from cold diethyl ether, dried and isolated as a yellowish solid, which was used without further purification (736 mg, 1.79 mmol, 98%). Isolated compound **7** by this method B is identical to that obtained by the method A.

#### Preparation of aniline hydrochlorides **8** and **16**. General procedure

The free amine was re-dissolved in  $\text{Et}_2\text{O}$  and a 1 N solution of HCl in  $\text{Et}_2\text{O}$  was added dropwise until an abundant precipitate was formed. The precipitate was filtered off, gently washed with additional amounts of  $\text{Et}_2\text{O}$  and then efficiently dried to give a solid that did not need further purification. Hydrochlorides **8** and **16** can be stored without noticeable degradation for months if kept in a cool and dry place.

**2,3,4-Tris(benzyloxy)aniline hydrochloride (8).** Following the general procedure, aniline **7** was converted into its hydrochloride **8**, isolated as a pale brownish solid. Mp 174.9–176.2 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 10.0 (br s, 3 H), 7.5 (d,  $J$  = 4.2 Hz, 2 H), 7.4–7.2 (m, 11 H), 7.2 (br d,  $J$  = 9.4 Hz, 1 H), 7.1 (dd,  $J$  = 5.1, 1.6 Hz, 2 H), 6.6 (d,  $J$  = 9.0 Hz, 1 H), 5.3 (s, 2 H), 5.1 (s, 2 H), 5.0 (s, 2 H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 152.8, 146.0, 142.2, 136.8, 136.6, 136.3, 128.7, 128.6, 128.3, 128.2, 127.9, 127.5, 118.9, 118.3, 109.3, 75.9, 75.6, 71.2;  $m/z$  (ESI $^+$ ): 823 ([ $2\text{M} - \text{HCl} + \text{H}$ ] $^+$ , 30%), 413 ([ $\text{M} - \text{HCl} + 2\text{H}$ ] $^+$ , 38%), 412 ([ $\text{M} - \text{HCl} + \text{H}$ ] $^+$ , 100%).

#### PyBOP-mediated synthesis of multivalent amides **13a–15a** and **17a**. General procedure

To a suspension of the suitable polyacid (1 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (20 mL  $\text{mmol}^{-1}$ ) at 0 °C (ice-bath) was added sequentially PyBOP (1.2 equiv. per acid group) and  $\text{Et}_3\text{N}$  (3.75 equiv. per acid group) in a pressure tube. When the acid is solubilized (approx. 5 min), aniline hydrochloride **8** (1.2 equiv. per acid group) was added and the pressure tube sealed. The reaction mixture was warmed to 60 °C and stirred overnight until consumption of the starting material (TLC monitoring). Then, reaction was cooled to room temperature, washed with a 10% citric acid aqueous solution and then with a 10%  $\text{NaHCO}_3$  aqueous solution, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under vacuum. The crude product was purified as determined.

**Tetrakis[5-oxo-5-[(2,3,4-tribenzyloxyphenyl)amino]-2-oxapentyl]-methane (13a).** A mixture of tetra-acid **10**<sup>38</sup> (21 mg, 0.050 mmol), hydrochloride **8** (108 mg, 0.240 mmol), PyBOP (126 mg, 0.240 mmol) and  $\text{Et}_3\text{N}$  (104  $\mu\text{L}$ , 0.725 mmol) in  $\text{CH}_2\text{Cl}_2$  (1 mL) was treated as described in the general procedure (reaction proceeded at room temperature). The crude product was purified by CCTLC (EtOAc-hexane 10–80%) to afford **13a** (58 mg, 0.029 mmol, 58%) as a yellowish waxy foam after freeze-drying. TLC ( $R_f$  = 0.26 hexane-EtOAc (2 : 3) v/v; CAM);  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$ : 7.80 (br s, 4 H), 7.75 (d,  $J$  = 9.3 Hz, 4 H), 7.41–7.27 (m, 60 H), 6.57 (d,  $J$  = 9.4 Hz, 4 H), 5.04 (s, 16 H), 5.01 (s, 8 H), 3.44 (t,  $J$  = 5.7 Hz, 8 H), 3.18 (s, 8 H), 2.26 (t,  $J$  = 5.7 Hz, 8 H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$ : 169.2, 149.2, 142.6, 141.5, 137.4, 137.0, 136.8, 128.7, 128.6, 128.5, 128.3, 128.0, 127.9, 127.5, 126.1, 115.7, 109.4, 76.1, 75.5,





71.2, 69.2, 67.0, 45.0, 37.7;  $m/z$  (ESI<sup>+</sup>) 1000 ([1/2M + 2H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>) [M + H]<sup>+</sup> C<sub>125</sub>H<sub>121</sub>N<sub>4</sub>O<sub>20</sub> requires 1997.8574, found: 1997.8568; Anal. calcd for C<sub>125</sub>H<sub>120</sub>N<sub>4</sub>O<sub>20</sub> (1996.3): C, 75.13; H, 6.05; N, 2.80; found: C, 74.93; H, 6.13; N, 2.95%.

The synthesis of compounds **14a–15a** and **17a** was performed similarly following the general procedure. For full details, see ESI<sup>†</sup> available.

#### Preparation of carbamates **28a–31a** and ureas **32a–33a** by Curtius rearrangement. General procedure

To a solution of the appropriate benzoic acid (1 equiv.) in toluene (5 mL mmol<sup>-1</sup>) under argon was added DPPA (1.1 equiv.) and Et<sub>3</sub>N (1.1 equiv.) at room temperature. The mixture was heated at 75 °C until complete disappearance of the starting material (as monitored by TLC), yielding the isocyanate intermediate **4** (not isolated). Then, the corresponding alcohols or amines were added (1.2 equiv. per isocyanate group to be functionalised). The reaction was stirred additionally at 75 °C overnight (monitored by TLC), cooled to room temperature and subsequently washed with a 1 N aqueous solution of HCl and then with a saturated aqueous solution of NaHCO<sub>3</sub>, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure. The crude product was purified as determined.

**Methyl (2,3,4-tris(benzyloxy)phenyl)carbamate (6a).** According to the general procedure, acid **3**<sup>33</sup> (1.55 g, 3.5 mmol), DPPA (830 μL, 4 mmol) and Et<sub>3</sub>N (540 μL, 4 mmol) in toluene (18 mL) were reacted to form isocyanate intermediate **4**, which was treated with methanol (170 μL, 0.4 mmol). After work-up, the crude mixture was purified by CCTLC (polar gradient on elution using EtOAc–hexane from (5 : 95) up to (20 : 80)) and further recrystallized from MeOH to yield **6a** (1.22 g, 2.6 mmol, 74%) as a white solid. Mp 89.1–90.7; TLC ( $R_f$  = 0.34 hexane–EtOAc (4 : 1) v/v; CAM); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 7.7 (br d, 1 H), 7.4–7.3 (m, 15 H), 6.89 (br s, 1 H), 6.76 (d,  $J$  = 9.2 Hz, 1 H), 5.09 (s, 4 H), 5.08 (s, 2 H), 3.71 (s, 3 H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ: 154.0, 148.4, 141.7, 137.3, 137.0, 136.8, 128.6, 128.5, 128.4, 128.3, 128.1, 127.9, 127.5, 126.2, 113.4, 109.8, 75.8, 75.6, 71.4, 52.2;  $m/z$  (ESI<sup>+</sup>) 492 ([M + Na]<sup>+</sup>, 59%), 470 ([M + H]<sup>+</sup>, 100%); Anal. calcd for C<sub>29</sub>H<sub>27</sub>NO<sub>5</sub> (469.5): C, 74.18; H, 5.80; N, 2.98; found: C, 74.27; H, 6.02; N, 3.20%.

**Tetrakis[5-(2,3,4-tribenzyloxyphenyl)carbamoyloxy-2-oxapentyl]methane (28a).** Following the general procedure, a solution of acid **3**<sup>33</sup> (478 mg, 1.1 mmol), DPPA (0.26 mL, 1.2 mmol) and Et<sub>3</sub>N (0.17 mL, 1.2 mmol) in toluene (5.4 mL) was reacted to form the isocyanate intermediate **4**, which was treated with tetraol **24** (100 mg, 0.27 mmol) (stirred overnight at 75 °C after addition of **24**). Additional acid **3** (239 mg, 0.54 mmol), DPPA (0.13 mL, 0.6 mmol) and Et<sub>3</sub>N (83 mL, 0.6 mmol) were needed for the reaction to proceed to completion. After the usual work-up, the crude material was purified by CCTLC (polar gradient EtOAc–hexane from (10 : 90) up to (50 : 50)) to afford **28a** (100 mg, 0.05 mmol, 21%) as a yellowish oil. TLC ( $R_f$  = 0.4 hexane–EtOAc (2 : 3) v/v; CAM); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)

δ: 7.66 (br s, 4 H), 7.42–7.29 (m, 60 H), 6.91 (br s, 4 H), 6.71 (d,  $J$  = 9.3 Hz, 4 H), 5.06 (s, 8 H), 5.05 (s, 8 H), 5.04 (s, 8 H), 4.16 (t,  $J$  = 6.4 Hz, 8 H), 3.47 (t,  $J$  = 6.3 Hz, 8 H), 3.42 (s, 8 H), 1.87 (qn,  $J$  = 6.3 Hz, 8 H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ: 153.5, 148.3, 141.8, 141.7, 137.4, 137.0, 136.9, 128.6, 128.5, 128.4, 128.3, 128.0, 127.9, 127.5, 126.3, 113.6, 109.8, 75.9, 75.5, 71.4, 69.7, 67.6, 62.4, 45.3, 29.2; HRMS (ESI<sup>+</sup>) [M + Na]<sup>+</sup> C<sub>129</sub>H<sub>128</sub>N<sub>4</sub>O<sub>24</sub>Na requires 2139.8816, found: 2139.8811.

The synthesis of carbamates **29a–31a** and ureas **32a–33a** was performed similarly following the general procedure. For full experimental details, see ESI<sup>†</sup> available.

#### Preparation of 1,2,3-triazole-bridged compounds **40a** and **43a** by copper-catalysed 1,3-dipolar azide–alkyne cycloaddition (Huisgen reaction). General procedure

To a suspension of the corresponding azide (1 equiv. per alkyne) and alkyne (1 equiv. per azide) in *t*BuOH–H<sub>2</sub>O (2 : 1) v/v (64 mL mmol<sup>-1</sup>) at room temperature was added (+)-sodium *L*-ascorbate (0.4 equiv.) and CuSO<sub>4</sub> (0.04 equiv.). The heterogeneous mixture was stirred at room temperature overnight and then treated with H<sub>2</sub>O. The resulting solid was filtered, washed with H<sub>2</sub>O and purified as determined.

**Tetrakis[5-(4-(2,3,4-tribenzyloxyphenyl)-1H-1,2,3-triazol-1-yl)-2-oxapentyl]methane (40a).** A suspension of tetra-azide **27** (20 mg, 0.04 mmol), alkyne **39** (72 mg, 0.17 mmol), (+)-sodium *L*-ascorbate (3 mg, 0.017 mmol) and CuSO<sub>4</sub> (1 mg, 0.002 mmol) in *t*BuOH–H<sub>2</sub>O (2.8 mL) was treated as described in the general procedure. The crude solid was purified by CCTLC on silica gel (polar gradient on elution, EtOAc–hexane from (40 : 60) up to (100 : 0) v/v) affording **40a** (75 mg, 0.04 mmol, 81%) as a yellowish oil. TLC ( $R_f$  = 0.32 hexane–EtOAc (3 : 7) v/v; PMA); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ: 7.89 (d,  $J$  = 8.8 Hz, 4 H), 7.74 (s, 4 H), 7.44–7.28 (m, 60 H), 6.84 (d,  $J$  = 8.9 Hz, 4 H), 5.10 (s, 8 H), 5.08 (s, 8 H), 5.03 (s, 8 H), 4.31 (t,  $J$  = 7.0 Hz, 8 H), 3.35 (t,  $J$  = 5.7 Hz, 8 H), 3.33 (s, 8 H), 1.97 (qn,  $J$  = 6.4 Hz, 8 H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ: 152.8, 149.9, 143.1, 141.8, 137.5, 137.4, 136.7, 128.6, 128.5, 128.3, 128.1, 128.0, 127.5, 122.5, 122.0, 118.4, 109.9, 75.6, 75.5, 70.9, 69.5, 67.6, 47.2, 45.4, 30.3; HRMS (ESI<sup>+</sup>) [M + H]<sup>+</sup> C<sub>133</sub>H<sub>129</sub>N<sub>12</sub>O<sub>16</sub> MH requires 2149.9650, found: 21.9644.

**Tetrakis[3-(1-(2,3,4-tribenzyloxyphenyl)-1H-1,2,3-triazol-4-yl)-2-oxapropyl]methane (43a).** A suspension of tetra-alkyne **42**<sup>54</sup> (14 mg, 0.048 mmol), azide **41** (83 mg, 0.190 mmol), (+)-sodium *L*-ascorbate (4 mg, 0.019 mmol) and CuSO<sub>4</sub> (1 mg, 0.002 mmol) in *t*BuOH–H<sub>2</sub>O (3.0 mL) was treated as described in the general procedure. Additional (+)-sodium *L*-ascorbate (0.4 equiv.) and CuSO<sub>4</sub> (0.04 equiv.) were added to ensure full conversion of the reaction. The crude solid was purified by CCTLC (EtOAc–hexane 20–100%) to afford **43a** (57 mg, 0.028 mmol, 60%) as a yellowish solid. Mp 50.2–51.1 °C; TLC ( $R_f$  = 0.30 hexane–EtOAc (3 : 7) v/v; PMA); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 7.78 (d,  $J$  = 2.7 Hz, 4 H), 7.43–7.30 (m, 32 H), 7.30–7.25 (m, 10 H), 7.21 (dd,  $J$  = 9.0, 4.6 Hz, 4 H), 7.18–7.14 (m, 10 H), 7.05–6.98 (m, 8 H), 6.76 (d,  $J$  = 9.1 Hz, 4 H), 5.08 (s, 8 H), 5.06 (s, 8 H), 4.87 (s, 8 H), 4.54 (s, 8 H), 3.52 (s, 8 H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ: 153.8, 146.1, 145.0, 142.7, 137.2,



136.4, 136.3, 128.8, 128.7, 128.5, 128.4, 128.3, 127.7, 125.6, 124.8, 120.5, 109.4, 76.4, 75.9, 71.3, 69.5, 65.2, 45.7; HRMS (ESI<sup>+</sup>) [M + H]<sup>+</sup> C<sub>125</sub>H<sub>113</sub>N<sub>12</sub>O<sub>16</sub> requires 2037.8398, found: 2037.8392; Anal. calcd for C<sub>125</sub>H<sub>112</sub>N<sub>12</sub>O<sub>16</sub> (2038.30): C, 73.66; H, 5.54; N, 8.25; found: C, 73.58; H, 5.51; N, 8.19%.

### Preparation of free polyphenols **6b**, **13b–15b**, **17b–21b**, **23b**, **28b–33b**, **37–38b/38c**, **40b** and **43b**. Deprotection of benzylated phenols by catalytic hydrogenation. General procedure

A solution of the corresponding benzyl-protected precursors (1 mmol) in a binary mixture of solvents THF–methanol (1 : 1) v/v (100 mL) containing catalytic amounts of Pd (on charcoal; 10 wt%) was hydrogenated overnight in a Parr Hydrogenator at 3.1 bar in a thermostated vessel at 25 °C. The Pd catalyst was removed by filtration through a Whatman® filter paper 42 and the solvent was removed under reduced pressure to give the corresponding deprotected free-phenolic derivatives as single products which were treated as specified. All debenzylated compounds should be kept at –20 °C and protected from direct exposure to light.

**Tetrakis[5-oxo-5-((2,3,4-trihydroxyphenyl)amino)-2-oxapentyl]-methane (13b)**. A solution of **13a** (50 mg, 0.025 mmol) in THF–MeOH (2.5 mL) was treated as described in the general procedure to afford **13b** (24 mg, 0.026 mmol, quantitative yield) as a yellowish foam after freeze-drying without further purification. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ: 6.70 (d, *J* = 8.6 Hz, 4 H), 6.32 (d, *J* = 9.0 Hz, 4 H), 3.59 (t, *J* = 5.7 Hz, 8 H), 3.42 (s, 8 H), 2.50 (t, *J* = 5.7 Hz, 8 H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ: 173.7, 145.2, 140.0, 136.0, 120.4, 114.6, 108.1, 71.0, 68.7, 46.9, 38.2; *m/z* (ESI<sup>+</sup>) 917 ([M + H]<sup>+</sup>, 100%), 477 ([M/2 + NH<sub>4</sub>]<sup>+</sup>, 94%); HRMS (ESI<sup>+</sup>) [M + Na]<sup>+</sup> C<sub>41</sub>H<sub>48</sub>N<sub>4</sub>NaO<sub>20</sub> requires 939.2760, found: 939.2749; Anal. calcd for C<sub>41</sub>H<sub>48</sub>N<sub>4</sub>O<sub>20</sub> (916.8): C, 53.71; H, 5.28; N, 6.11; found: C, 53.42; H, 5.58; N, 6.31%; HPLC: *t*<sub>R</sub> = 2.86 min. polar gradient CH<sub>3</sub>CN–H<sub>2</sub>O from (10 : 90) up to (100 : 0) in 5 min (96% purity).

The synthesis of compounds **6b**, **14b–15b**, **17b–21b**, **23b**, **28b–33b**, **37b–38b/38c**, **40b** and **43b** was performed similarly following the general procedure. For full details, see ESI.†

### Antiviral activity

The methodology of the anti-HIV assays was as follows: human CD<sub>4</sub><sup>+</sup> T-lymphocyte CEM cells (~3 × 10<sup>5</sup> cells mL<sup>-1</sup>) were infected with 100 CCID<sub>50</sub> of HIV-1(IIIB) or HIV-2(ROD) mL<sup>-1</sup> and seeded in 200 μL wells of a 96-well microtiter plate containing appropriate dilutions of the test compounds. After 4 days of incubation at 37 °C, HIV-induced CEM giant cell formation was examined microscopically. The 50% effective concentration (EC<sub>50</sub>) was defined as the compound concentration required to inhibit virus-induced cytopathy by 50%. The 50% cytostatic concentration (CC<sub>50</sub>) was defined as the compound concentration required to inhibit CEM cell proliferation by 50% as counted by a Coulter counter.

## Acknowledgements

This work has been supported by the Spanish MICINN/MINECO (projects SAF2009-13914-C02-01 and SAF2012-39760-C02-01), Comunidad de Madrid (BIPEDD-2 project CM-S2010/BMD-2457) and the KU Leuven (GOA no. 10/014; EF 05/15; PF 10/018). Dr A. Flores acknowledges financial support from a contract JAE-Doc (Programa Junta para la Ampliación de Estudios) co-funded by CSIC and UE (FSE, Fondo Social Europeo). Sonsoles Velázquez is acknowledged for critical reading and valuable comments on this article and Leen Ingels for dedicated technical assistance with the antiviral assays.

## References

- UNAIDS report on the global AIDS epidemic 2013. Published November 18, 2013. [http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS\\_Global\\_Report\\_2013\\_en.pdf](http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf) (accessed February 15th, 2014).
- (a) An up-to-date list of approved drugs against HIV infections is available at <http://www.fda.gov> (accessed February 15th, 2014); (b) E. De Clercq, *Curr. Opin. Pharmacol.*, 2010, **10**, 507.
- S. T. Butera, in *HIV Chemotherapy: A Critical Review*, Caister Academic Press, Norwich, 2005.
- R. J. Pomerantz and D. L. Horn, *Nat. Med.*, 2003, **9**, 867.
- L. Menendez-Arias, *Antiviral Res.*, 2013, **98**, 93.
- T. Hawkins, *Antiviral Res.*, 2010, **85**, 201.
- (a) A. Lefeullade, *Curr. HIV/AIDS Rep.*, 2012, **9**, 121; (b) T.-W. Chun and A. S. Fauci, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 10958.
- (a) S. G. Deeks, D. Autran, B. Berkhout, M. Benkirane, S. Cairns, N. Chomont, T.-W. Chun, M. Churchill, M. Di Mascio, C. Katlama, A. Lefeullade, A. Landay, M. Lederman, S. R. Lewin, F. Maldarelli, D. Margolis, M. Markowitz, J. Martinez-Picado, J. I. Mullins, J. Mellors, S. Moreno, U. O'Doherty, S. Palmer, M.-C. Penicaud, M. Peterlin, G. Poli, J. P. Routy, C. Rouzioux, G. Silvestri, M. Stevenson, A. Telenti, C. Van Lint, E. Verdin, A. Woolfrey, J. Zaia and F. Barré-Sinoussi, *Nat. Rev. Immunol.*, 2012, **12**, 607; (b) S. Broder, *Sci. Transl. Med.*, 2010, **2**, 39ps33.
- V. Lattanzio, P. A. Kroon, S. Quideau and D. Treutter, Plant phenolics: secondary metabolites with diverse functions, in *Recent Advances in Polyphenol Research*, ed. F. Daayf and V. Lattanzio, Wiley-Blackwell, Oxford, UK, 2008, vol. 1, pp. 1–34.
- For recent reviews on polyphenols and HIV infection, see: (a) K. Andrae-Marobela, F. W. Ghislain, H. Okatch and R. R. Majinda, *Curr. Drug Metab.*, 2013, **14**, 392; (b) S. Yu and G. Zhao, *Curr. Med. Chem.*, 2012, **19**, 5536. For a general review on polyphenols, see: (c) S. Quideau, D. Deffieux, C. Douat-Casassus and L. Pouysegu, *Angew. Chem., Int. Ed.*, 2011, **50**, 586.



- 11 Y. Zhao, F. Jiang, P. Liu, W. Chen and K. Yi, *Drug Discovery Today*, 2012, **17**, 6930.
- 12 J. Yang, L. Li, S. Tan, H. Jin, J. Qiu, Q. Mao, R. Li, C. Xia, Z. H. Jiang, S. Jiang and S. Liu, *Fitoterapia*, 2012, **83**, 348.
- 13 J. Yang, L. Li, H. Jin, S. Tan, J. Qiu, L. Yang, Y. Ding, Z. H. Jiang, S. Jiang and S. Liu, *AIDS Res. Hum. Retroviruses*, 2012, **28**, 1498.
- 14 (a) Y. B. Yu, H. Miyashiro, N. Nakamura, M. Hattori and J. C. Park, *Arch. Pharmacol. Res.*, 2007, **30**, 820; (b) M. P. N. Nair, Z. M. Saiyed, N. H. Gandhi and C. N. Ramchand, *Am. J. Infect. Dis.*, 2009, **5**, 135.
- 15 (a) M. Jourdes, L. Pouységou, D. Deffieux, P.-L. Teissedre and S. Quideau, Hydrolysable Tannins: Gallotannins and Ellagitannins, in *Natural Products*, ed. K. Gopal Ramawat and J.-M. Merillon, Springer-Verlag, Berlin, 2013, pp. 1975–2010; (b) P. Buzzini, P. Arapitsas, M. Goretti, E. Branda, B. Turchetti, P. Pinelli, F. Ieri and A. Romani, *Mini-Rev. Med. Chem.*, 2008, **8**, 1179; (c) I. Mueller-Harvey, *Anim. Feed Sci. Technol.*, 2001, **91**, 3.
- 16 K. Takada, A. Birmingham, B. R. O'Keefe, A. Wamiru, J. A. Beutler, S. F. Le Grice, J. Lloyd, K. R. Gustafson and J. B. McMahon, *J. Nat. Prod.*, 2007, **70**, 1647.
- 17 (a) X. Wang, K. S. Song, Q. X. Guo and W. X. Tian, *Biochem. Pharmacol.*, 2003, **66**, 2039; (b) C. Braicu, M. R. Lodomery, V. S. Chedea, A. Irimie and I. Berindan-Neagoe, *Food Chem.*, 2013, **141**, 3282.
- 18 (a) E. Q. Xia, G. F. Deng, Y. J. Guo and H. B. Li, *Int. J. Mol. Sci.*, 2010, **11**, 622; (b) J. Intra and S. M. Kuo, *Chem.-Biol. Interact.*, 2007, **169**, 91.
- 19 O. Takahashi, Y. Kohno and M. Nishio, *Chem. Rev.*, 2010, **110**, 6049.
- 20 C. S. Yang, X. Wang, G. Lu and S. C. Picinich, *Nat. Rev. Cancer*, 2009, **9**, 429.
- 21 (a) N. Israëil and M. A. Gougerot-Pocidallo, *Cell Mol. Life Sci.*, 1997, **53**, 864; (b) H. C. Greenspan and O. I. Aruoma, *Immunol. Today*, 1994, **15**, 209.
- 22 S. Coaccioli, G. Crapa, M. Fantera, R. Del Giorno, A. Lavagna, M. L. Standoli, R. Frongillo, R. Biondi and A. Puxeddu, *Clin. Ther.*, 2010, **161**, 55.
- 23 M. A. Edeas and A. Lindenbaum, *Bull. l'O.I.V.*, 2000, **73**, 810.
- 24 F. Nanjo, M. Mori, K. Goto and Y. Hara, *Biosci., Biotechnol. Biochem.*, 1999, **63**, 1621.
- 25 (a) L. L. Kiessling, J. E. Gestwicki and L. E. Strong, *Curr. Opin. Chem. Biol.*, 2000, **4**, 696; (b) C. Fasting, C. A. Schalley, M. Weber, O. Seitz, S. Hecht, B. Kokschi, J. Dermedde, C. Graf, E. W. Knapp and R. Haag, *Angew. Chem., Int. Ed.*, 2012, **51**, 10472.
- 26 Y. Song, P. Zhan, X. Li, D. Rai, E. De Clercq and X. Liu, *Curr. Med. Chem.*, 2013, **20**, 815.
- 27 V. Lozano, L. Aguado, B. Hoorelbeke, M. Renders, M. J. Camarasa, D. Schols, J. Balzarini, A. San-Félix and M. J. Pérez-Pérez, *J. Med. Chem.*, 2011, **54**, 5335.
- 28 (a) P. J. King, G. Ma, W. Miao, Q. Jia, B. R. McDougall, M. G. Reinecke, C. Cornell, J. Kuan, T. R. Kim and W. E. Robinson Jr., *J. Med. Chem.*, 1999, **42**, 497; (b) C. O. R. Junior, S. C. Verde, C. A. M. Rezende, W. Caneschi, M. R. C. Couri, B. R. McDougall, W. E. Robinson Jr. and M. V. de Almeida, *Curr. Med. Chem.*, 2013, **20**, 724.
- 29 P. Carrero, A. Ardá, M. Alvarez, E. G. Doyagüez, E. Rivero-Buceta, E. Quesada, A. Prieto, D. Solís, M.-J. Camarasa, M.-J. Pérez-Pérez, J. Jiménez-Barbero and A. San-Félix, *Eur. J. Org. Chem.*, 2013, 65–76.
- 30 (a) J. Katajisto, P. Heinonen and H. Loennberg, *Curr. Org. Chem.*, 2004, **8**, 977; (b) G. Newkome, J. K. Young, G. R. Baker, R. L. Potter, L. Audoly, D. Cooper and C. D. Weis, *Macromolecules*, 1993, **26**, 2394.
- 31 *Hydrolysis in Drug and Prodrug Metabolism: Chemistry, Biochemistry, and Enzymology*, ed. B. Testa and J. M. Mayer, Verlag Helvetica Chimica Acta, Wiley-VCH, Zurich, 2003, ch. 4, pp. 81–126.
- 32 F. Belin, P. Barthélémy, K. Ruiz, J. M. Lacombe and B. Pucci, *Helv. Chim. Acta*, 2003, **86**, 247.
- 33 (a) N. Takashi, K. Kumiko, A. Akiko, M. Kenji and T. Koichiro, *Jp. Pat* JP8143525 (A), 1996; (b) S. Lee, S. Hwang, S. Yu, W. Jang, Y. M. Lee and S. Kim, *Arch. Pharm. Res.*, 2011, **34**, 1065.
- 34 P. A. S. Smith, *Org. React.*, 1946, **3**, 337.
- 35 T. Shioiri, K. Ninomiya and S. Yamada, *J. Am. Chem. Soc.*, 1972, **94**, 6203.
- 36 A. Maiti, P. V. N. Reddy, M. Sturdy, L. Marler, S. D. Pegan, A. D. Mesecar, J. M. Pezzuto and M. Cushman, *J. Med. Chem.*, 2009, **52**, 1873.
- 37 U. Jacquemard, V. Bénéteau, M. Lefoix, S. Routier, J. Y. Mérou and G. Coudert, *Tetrahedron*, 2004, **60**, 10039.
- 38 G. Newkome and C. D. Weis, *Org. Prep. Proced. Int.*, 1996, **28**, 242.
- 39 (a) A. Dupraz, P. Guy and C. Dupuy, *Tetrahedron Lett.*, 1996, **37**, 1237; (b) *tert*-Butyl ester intermediate was isolated by high-vacuum distillation ( $10^{-2}$  mbar; b.p. ranging 135–150 °C) in 73% yield.
- 40 S. Aime, C. Cavallotti, G. Cravotto, G. B. Giovenzana and G. Palmisano, *Tetrahedron Lett.*, 2004, **45**, 5901.
- 41 (a) M. W. Wilson and J. C. Hodges, *Org. Prep. Proced. Int.*, 1993, **25**, 665; (b) Intermediate dinitrile was isolated by high-vacuum distillation ( $10^{-2}$  mbar; bp ranging 120–130 °C) in 98% yield.
- 42 G. R. Newkome and X. Li, *Macromolecules*, 1991, **24**, 1443.
- 43 G. R. Newkome and E. He, *J. Mater. Chem.*, 1997, **7**, 1237.
- 44 Y. Sun and A. E. Martell, *J. Am. Chem. Soc.*, 1989, **111**, 8023.
- 45 S. B. Fredriksen and J. Dale, *Acta Chem. Scand.*, 1992, **46**, 574.
- 46 G. R. Newkome, A. Mishra and C. N. Moorefield, *J. Org. Chem.*, 2002, **67**, 3957.
- 47 C. K. Luscombe, S. Proemmel, W. T. S. Huck, A. B. Holmes and H. Fukushima, *J. Org. Chem.*, 2007, **72**, 5505.
- 48 B. A. Horestein and K. Nakanishi, *J. Am. Chem. Soc.*, 1989, **111**, 6242.
- 49 C. A. Hansen, A. B. Dean, K. M. Draths and J. W. Frost, *J. Am. Chem. Soc.*, 1999, **121**, 3799.



- 50 H. J. Wadsworth, S. M. Jenkins, B. S. Orlek, F. Cassidy, M. S. G. Clark, F. Brown, G. J. Riley, D. Graves, J. Hawkins and C. B. Naylor, *J. Med. Chem.*, 1992, **35**, 1280.
- 51 (a) E. Quesada and R. J. K. Taylor, *Tetrahedron Lett.*, 2005, **46**, 6473; (b) E. Quesada, S. A. Raw, M. Reid, S. Roman and R. J. K. Taylor, *Tetrahedron*, 2006, **62**, 6673. For a review on *Tandem Oxidation Processes* (TOP), see: (c) R. J. K. Taylor, M. Reid, J. Foot and S. A. Raw, *Acc. Chem. Res.*, 2005, **38**, 851.
- 52 For a review on conversion of carbonyl compounds into alkynes, see: D. Habrant, V. Rauhala and A. M. P. Koskinen, *Chem. Soc. Rev.*, 2010, **39**, 2007.
- 53 G. C. Tron, T. Pirali, R. A. Billington, P. L. Canonico, G. Sorba and A. A. Genazzani, *Med. Res. Rev.*, 2008, **28**, 278.
- 54 (a) F. G. Calvo-Flores, J. Isac-Garcia, F. Hernández-Mateo, F. Pérez-Balderas, J. A. Calvo-Asín, E. Sánchez-Vaquero and F. Santoyo-González, *Org. Lett.*, 2000, **2**, 2499; (b) A. Natarajan, W. Du, C. Y. Xiong, G. L. DeNardo, S. J. DeNardo and J. Gervay-Hague, *Chem. Commun.*, 2007, **7**, 695.
- 55 D. D. Perrin, W. L. F. Armarego and D. R. Perrin, in *Purification of Laboratory Chemicals*, Pergamon Press, Oxford, 2nd edn, 1980.
- 56 W. C. Still, M. Kahn and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923.

