First Class of Phosphorus Dendritic Compounds Containing β-Cyclodextrin Units in the Periphery Prepared by CuAAC

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Abstract: A new class of phosphorus dendritic compounds (PDCs) having a cyclotriphosphazene (P3N3) core and decorated with six β-cyclodextrin (βCD) units, named P3N3-[O-C6H4-O-(CH2)n-βCD]6, where n = 3 or 4 was designed, and the synthesis was performed using copper (I) catalyzed alkyne-azide cycloaddition (CuAAC). To obtain the complete substitution of the P3N3, two linkers consisting of an aromatic ring and an aliphatic chain of two different lengths were assessed. We found that, with both linkers, the total modification of the periphery was achieved. The two new obtained dendritic compounds presented a considerably high water solubility (>1 g/mL). The compounds comprised in this new class of PDCs are potential drug carrier candidates, since the conjugation of the βCD units to the P3N3 core through the primary face will not only serve as surface cover but, also, provide them the faculty to encapsulate various drugs inside the βCDs cavities.

Keywords: phosphorus dendritic compounds; β-cyclodextrin; CuAAC reaction

1. Introduction

The use of nanomedicine confers a substantial potentiality for drug delivery and targeted release, as it increases the safety by reducing toxic effects in nontargeted organs and tissues [1–3]. In recent years, many researchers have focused on the development of dendrimers, since they have distinctive properties, such as monodispersity, a large number of easily available functional groups on the surface and an extraordinary capability to encapsulate host molecules within their hydrophobic environment, making them ideal nanocarriers for the targeted delivery (with or without ligand) of therapeutic and diagnostic agents [4–6].

Dendrimers are well-defined hyper-branched three-dimensional macromolecules. Structurally, dendrimers have a core from which branches (or arms) are derived and end with multiple peripheral groups that determine their macroscopic properties [7]. Dendrimers can be constructed starting from the core towards the periphery (divergent synthesis) or through a top-down approach, from the
appropriate outer residues (convergent synthesis) [8]. It is clear that, when designing a new dendrimer, the chemical composition of the core, branches and peripheral groups will have crucial implications for enhancing possible biomedical applications, either by themselves or as nanocarriers [9].

Among the diverse existing types of dendrimers, phosphorus containing dendrimers, (i.e., dendrimers having phosphorus derivatives in their structure) play a special role in applications such as drug delivery, gene therapy and biomaterials, among others [10,11].

In 1994, Caminade et al. described the first method for the synthesis of high-generation phosphorus dendrimers (PDs), which is still the most widely used, since it allows numerous changes at the level of the core, branches or terminal groups, conferring biocompatible functions for the use in biological applications [12]. In this way, PD synthesis can be started from different cores; however, hexachlorocyclotriphosphazene (P₃N₃Cl₆) is the most used core, since it provides twice the number of final groups, compared with thio phosphoryl chloride (P(S)Cl₃) [10]. Using the P₃N₃Cl₆ core, modifications to the terminal groups of the PDs can be achieved with different molecules in order to improve the biocompatible properties. One of the strategies used is the conjugation with different types of carbohydrates to improve the aqueous solubility and correlate the structures with the impacts on biological behaviors [13,14]. For instance, the construction of PDs with mannose residues in its periphery was employed as a strategy for the treatment of lung inflammation [15]. Furthermore, PDs conjugated with mannopyranoside have been used as multivalent carbohydrate protein-binding recognition probes [16]. Finally, PDs were used as noncovalent carriers of aminolactitol carbohydrate for treatment against the HIV virus [17].

With the focus of developing carbohydrate-conjugated dendrimers, diverse modifications of poly(amidoamines) (PAMAM) dendrimers with cyclodextrin molecules (CDs) have been reported for multiple applications [18–21]; however, this strategy has not yet been reported for PDs. CDs are cyclic oligosaccharides, composed of six or more glucopyranose units linked by α (1-4)-glucosidic bonds. Typical native CDs contain six, seven or eight glucose units and are named α-, β- and γ-, respectively. Due to the lack of free rotations between glucose units, CDs present a truncated cone shape and a hydrophobic internal cavity. β-cyclodextrin (βCD) is the most studied and frequently used CD, due to its low cost, availability and ability to encapsulate a wide range of molecules in its internal cavity. The variable reactivity of the hydroxyl groups and the remarkable encapsulation properties that can modify and/or improve the physical, chemical and biological characteristics of host molecules make it the perfect candidate to be used as building blocks in the construction of drug delivery systems [22–24].

Synthesis mediated by copper-catalyzed azide-alkyne cycloaddition (CuAAC) to produce 1,4-disubstituted 1,2,3-triazoles has been demonstrated to be effective in the design of new dendrimers. CuAAC coupling confers high specificity, mild reaction conditions and quantitative synthetic yields [25]. The physicochemical properties of the triazole ring are particularly favorable when added to structures with biological applications, since triazole units act as rigid bonds. Unlike amides, the triazole ring cannot be hydrolytically cleaved, oxidized or reduced [26].

For all these reasons, in this work, we propose the design, synthesis and characterization of a first class of phosphorus dendritic compounds (PDCs) decorated with βCD units, using aromatic rings and aliphatic chains as spacers P₃N₃-[O-C₆H₄-O-(CH₂)ₙ-βCD]₆ (n = 3 or 4). We selected P₃N₃ as the core, and in the first step, alkyne groups were added to the periphery. Finally, the grafting of βCD units was carried out by the CuAAC reaction. The grafting of βCD units on its primary side will allow the secondary face to be available for the formation of inclusion complexes, so that these new PDCs are potential candidates as drug nanocarriers and may have applications in nanomedicine.

2. Results and Discussion

2.1. Synthesis

The synthetic route of intermediates HO-C₆H₄-O-(CH₂)ₙ-alkyne (where n = 3 or 4) is shown in Scheme 1. The intermediates A and B were prepared via a Williamson etherification reaction between
the hydroquinone and the respective X-alkynes (X = Cl for n = 3 and I for n = 4), using K₂CO₃ as a base and anhydrous N,N-dimethylformamide (DMF) as the solvent at 72 °C. Mono and difunctionalized products were obtained, and they could be separated by column chromatography. Subsequently, the yields of size exclusion chromatography, using water as the eluent, and were obtained in high purity and with 50%. We found that the length of the linker did not affect the complete functionalization.

The synthetic route of intermediates P₃N₃-[O-C₆H₄-O-(CH₂)ₙ-alkyne]₆ (n = 3 or 4) is shown in Scheme 1. The preparation of the intermediates was achieved, as previously reported in the literature but using phenols with a different substitution pattern [28]. The reaction proceeded through a substitution reaction of the Cl atoms attached to the P₃N₃ core; this substitution was carried out in basic and anhydrous medium using intermediates A and B to obtain compounds C and D, respectively.

The next step in the synthetic route was the formation of mOTs-βCD (E), followed by mN₃-βCD (F), which is shown in Scheme 1. For the synthesis of mOTs-βCD, the most efficient technique reported in the literature was employed to modify a single position of the native βCD [29], with minimal differences in the purification of the final product. It is worth noticing that this synthetic step was crucial in the design of our PDCs. In particular, the βCD tosylation method provided high yields compared to other previously reported methods [30,31]. Then, the tosyl group of compound E was replaced by an azide group through a nucleophilic substitution reaction to give compound F [32].

Once the necessary intermediates, alkynes (P₃N₃-[O-C₆H₄-O-[CH₂]ₙ-alkyne]₆) and azide (mN₃-βCD) were obtained, the CuAAC reaction was carried out, as shown in Scheme 1. According to the design of these novel PDCs, we used two linkers with different lengths of the aliphatic chain to assess whether this factor had an influence on the complete functionalization of all positions of the P₃N₃ core. To carry out the CuAAC reaction, we used a Cu(I) catalyst synthesized in situ, with CuSO₄ as the copper source and H₂Asc as a reducing agent, using dimethylsulfoxide (DMSO) as the ideal solvent, since it solubilizes the azide and the respective alkynes. To ensure the complete functionalization of the six core groups of P₃N₃, an excess of mN₃-βCD was added. The final PDCs G and H were purified by size exclusion chromatography, using water as the eluent, and were obtained in high purity and with yields of >50%. We found that the length of the linker did not affect the complete functionalization.

Scheme 1. Synthesis of P₃N₃-[O-C₆H₄-O-(CH₂)ₙ-βCD]₆ (n = 3 or 4) phosphorus dendritic compounds (PDCs). Conditions: (i) K₂CO₃, X-alkyne, N,N-dimethylformamide (DMF) anhydrous, 72 °C, 36 h. (ii) (A,B), Cs₂CO₃, P₃N₃Cl₆, tetrahydrofuran anhydrous (THF) anhydrous, 7 days, room temperature (RT). (iii) TsO/NaOH, H₂O, 2 h, RT. (iv) NaN₃, DMF anhydrous, 80 °C, 48 h. (v) mN₃-βCD, CuSO₄•5H₂O, H₂Asc, dimethylsulfoxide (DMSO):H₂O (7:1), 80 °C, 7 days.
of P₃N₃ with the βCD units, since all six positions of the P₃N₃ core were functionalized with both proposed linkers.

### 2.2. Characterization

The full characterization of all the synthetic intermediates and final compounds was carried out. Regarding intermediates A and B, in the ¹H-NMR spectra (Figures S1 and S4 are available in Supplementary Materials (SM)) in DMSO-d₆, it is possible to observe the signal corresponding to the proton of the alkyne group at 2.80 and 2.78 ppm, respectively. Moreover, the signal of the phenol proton appeared between 8.90 and 8.88 ppm. The structure of intermediates A and B was also confirmed by the ¹³C-NMR spectra (Figures S2 and S5 are available in SM), in which the signals corresponding to the carbons of the alkyne group appeared at 84.56 and 72.34 ppm and at 83.42 and 72.19 ppm, respectively. The structure of these intermediates was also confirmed by mass spectrometry using the Direct Analysis in Real Time (DART) technique (Figures S3 and S6 are available in SM). The molecular ions appeared at 177 m/z (A) and 191 m/z (B), which correspond to the molecular weights of the proposed compounds.

The presence of phosphorus in the core allowed simple monitoring by ³¹P-NMR; furthermore, this allowed to verify the completion of the reactions in each synthesis step, as well as the integrity of the entire structure [33]. For the characterization of the intermediates C and D, a single signal corresponding to the complete functionalization was observed in the ³¹P-NMR spectrum (Figures S9 and S13 are available in SM). Furthermore, the complete functionalization of the phosphorus core was confirmed with the rest of the characterization. In the ¹H-NMR spectrum (Figures S7 and S11 are available in SM), the disappearance of the signal corresponding to the phenol proton present in intermediates A and B, as mentioned above, is clearly observed due to its grafting on P₃N₃. The structure of C and D was also confirmed by ¹³C-NMR (Figures S8 and S12 are available in SM) and Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry (Figures S10 and S14 ESI), where the molecular ions appeared at 1187.623 m/z and 1271.794 m/z, which correspond to the molecular weights of compounds C and D, respectively. The structures of the compounds P₃N₃-[O-C₆H₄-O-(CH₂)₆-alkyne]₆ (where n = 3 or 4) were confirmed by all the characterization data.

The full characterization of two new P₃N₃-[O-C₆H₄-O-(CH₂)₆-βCD]₆ (G) and (H) PDCs using NMR techniques (¹H-, ¹³C-NMR and 2D NMR Heteronuclear Multiple-Quantum Correlation (HMQC) and Homonuclear Correlation Spectroscopy (COSY)) (Figures S15–S17 and S19–S22 are available in SM) was carried out in DMSO-d₆. The classical signals of the linkers and native βCD units are in agreement with those reported in previous works [17,26]. Moreover, it was possible to observe the differentiation for some protons of the modified glucopyranose unit of the βCD (see Figure 1). The signals of protons H-1’, H-5’ and, particularly, H-6’ appeared at a lower field than those of H-1, H-5 and H-6 of the nonfunctionalized subunits, due to the change in their chemical environment after the CuAAC reaction. In the same way, it was possible to identify the H-6” diastereotopic protons, corresponding to one -CH₂-OH fragment contiguous to the substituted one at 3.12 and 2.94 ppm (see HMQC in Figure 2). Therefore, H-6” and the adjacent OH-6” on the primary face (around 4.33 ppm) appeared significantly upfield-shifted in comparison to their respective analogs H-6 and OH-6 because of the change in chemical environment due to the neighboring substitution. In this step, ³¹P-NMR was used to assess the completion of the reactions and to assure the complete functionalization of the six positions of P₃N₃. This was confirmed by the appearance of a single signal (at 9.31 ppm) in the ³¹P-NMR spectrum of P₃N₃-[O-C₆H₄-O-(CH₂)₆-βCD]₆ (where n = 3, compound G). This signal exhibited an upfield shift compared to the signal corresponding to P₃N₃-[O-C₆H₄-O-(CH₂)₆-alkyne]₆ (where n = 3, compound C) (at 9.93 ppm), because P₃N₃ is protected by βCD molecules (Figure 3). The same behavior was observed in the ³¹P-NMR spectrum (Figure S24 is available in SM) of P₃N₃-[O-C₆H₄-O-(CH₂)₆-βCD]₆ (where n = 4, compound H). Finally, the structures of these new dendrimers were corroborated by MALDI-TOF mass spectrometry (Figures S18 and S23 are available in SM). The molecular ions appeared mainly at 8169.59 m/z and at 8254.30 m/z, corresponding to the molecular weights of the PDCs G.
and H, respectively. Furthermore, the signals at 7034.596 m/z and 7120.029 m/z for compounds G and H, respectively, correspond to partial ionization, since complete ionization of the molecule is complex when using this technique. This difficulty has been previously reported for other phosphorus dendrimers [34].

**Figure 1.** Assignation of the protons in the NMR of P3N3-[O-C6H4-O-(CH2)3-βCD]6 dendritic compounds (PDCs).

**Figure 2.** 2D NMR Heteronuclear Multiple-Quantum Correlation (HMQC) spectra of P3N3-[O-C6H4-O-(CH2)3-βCD]6 PDCs in DMSO-d6.
3. Materials and Methods

3.1. General Notes

All starting materials were commercially available reagent grade and were used without any further purification. Hexachlorocyclotriphosphazene (P₃N₃Cl₆), 5-chloro-1-pentyne, 6-iodo-1-hexyne, hydroquinone, β-cyclodextrin (βCD), p-toluenesulfonyl chloride (Cl-Ts), p-toluenesulfonic acid (OH-Ts), N,N-dimethylformamide anhydrous (DMF), potassium carbonate (K₂CO₃), cesium carbonate (Cs₂CO₃), dimethylsulfoxide (DMSO), ascorbic acid (H₂Asc), Bio-Gel P-10 medium from Bio-Rad (Hercules, California, U.S.A.) were used.

2.3. Determination of Water Solubility for P₃N₃-[O-C₆H₄-O-(CH₂)₃-βCD]₆ PDCs

Among all the special properties of dendrimers, their high solubility makes an important difference compared to linear polymers. Differences in solubility between hyper-branched and linear polymers can be of several orders of magnitude, up to 10⁶ times, in some cases [35]. Among all the solvents used to dissolve dendrimers, water is especially important when considering biological applications. In recent years, the importance of water-soluble phosphorous-containing dendrimers has increased. In most cases, the water solubility of these compounds was dependent mainly on the reactivity of terminal groups in the periphery [36]. Therefore, the determination of the water solubility of our new PDCs was carried out, according to a method previously reported in the literature [37]. It was found that, for both PDCs, the solubility in water was >1g/mL. These results represent a considerably higher solubility compared to the solubility of native βCD (18.5 mg/mL) and of other commercial βCD derivatives, such as sulfobutylether-βCD (>500 mg/mL), O-methyl-βCD (>500 mg/mL) and 2-hydroxypropyl-βCD (>600 mg/mL) [38]. Water-soluble phosphorous-containing dendrimers with cationic or anionic end groups have been reported; nevertheless, their water solubility was dependent on the pH [17,35]. On the contrary, the advantage of our new P₃N₃-[O-C₆H₄-O-(CH₂)₃-βCD]₆ PDCs (n = 3 and 4) is that their solubility in water is conferred by the βCD units in standard conditions.
CA, USA), potassium iodide (KI), sodium azide (NaN₃), sodium hydroxide (NaOH), copper sulfate pentahydrate (CuSO₄•5H₂O), diisopropyl ether, dichloromethane (CH₂Cl₂), ethyl acetate (EtOAc),
tetrahydrofuran anhydrous (THF), hexane (HEX), methanol (MeOH), ethanol (EtOH) and acetone
(CH₃COCH₃) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

3.2. Synthetic Procedures

3.2.1. Synthesis of HO-C₆H₄-O-(CH₂)ₙ-alkyne (n = 3 and 4) A and B

The synthesis of the intermediates A and B was carried out according to a previously reported
procedure, with some modifications [27,28]. A dry hydroquinone solution (24.03 mmol) in DMF (250 mL)
was refluxed for 30 min at 72 °C, K₂CO₃ (30.04 mmol) was added and the mixture was refluxed for 1 h.
To this mixture, alkyne (12.02 mmol) was added dropwise over 2 h. The resulting mixture was refluxed
for 36 h, then cooled to 25 °C and filtered. The filtrate was evaporated under reduced pressure. The
resulting brown oil was dissolved in CH₂Cl₂ (150 mL), and the solution was extracted with water
(3 × 50 mL), the organic phase was dried with anhydrous Na₂SO₄ and the solvent was evaporated.
The crude product was composed of a mixture of unreacted hydroquinone, the monofunctionalized
and the difunctionalized products, which were separated by column chromatography on silica gel
using hexanes:EtOAc (8:2). Once the fraction corresponding to the monofunctionalized product was
obtained, it was recrystallized from hot/cold hexane, and the final product was recovered by filtration;
the solid was left to dry overnight under vacuum. HO-C₆H₄-O-(CH₂)₃-alkyne was obtained as a beige
solid (7.17 mmol, 29%). HO-C₆H₄-O-(CH₂)₄-alkyne was obtained as a yellow solid (7.88 mmol, 66%).

HO-C₆H₄-O-(CH₂)₃-alkyne. ¹H-NMR (400 MHz, DMSO-d₆, δ ppm): 8.90 (s, 1H, PhOH), 6.76 (d, J = 9 Hz,
2H, Ha), 6.68 (d, J = 9 Hz, 2H, Hb), 3.93 (t, J = 2.4 Hz, 2H, Hc), 2.80 (t, J = 2.4 Hz, 1H, C≡C-H),
2.31 (t, J = 2.4 Hz, 2H, Hc), 1.86 (t, J = 2.4 Hz, 2H, Hd); ¹³C-DEPTQ NMR (101 MHz, DMSO-d₆, δ ppm):
152.01, 116.50, 116.20, 84.56, 72.34, 67.15, 28.67, 15.29. DART-MS: 177 m/z (M+H)+; 178 m/z (M + 2H)+.

HO-C₆H₄-O-(CH₂)₄-alkyne. ¹H-NMR (400 MHz, DMSO-d₆, δ ppm): 8.88 (s, 1H, PhOH), 6.75 (d, J = 9 Hz,
2H, Ha), 6.67 (d, J = 9 Hz, 2H, Hb), 3.88 (t, J = 2.4 Hz, 2H, Hc), 2.78 (t, J = 2.4 Hz, 1H, C≡C-H),
2.22 (t, J = 2.4 Hz, 2H, Hd), 1.75 (t, J = 2.4 Hz, 2H, Hf), 1.58 (t, J = 2.4 Hz, 2H, He); ¹³C-DEPTQ NMR
(101 MHz, DMSO-d₆, δ ppm): 152.19, 151.88, 116.24, 116.14, 83.42, 72.19, 68.10, 28.75, 25.49, 18.26.
DART-MS: 191 m/z (M+H)+; 192 m/z (M + H)+.

3.2.2. Synthesis of P₃N₃-[O-C₆H₄-O-(CH₂)ₙ-alkyne]₆ (n = 3 and 4) C and D

The synthesis of intermediates C and D was carried out according to a previously reported
procedure, with some modifications [39]. A solution of intermediate A or B (2.84 mmol) in dry THF
(25 mL) was stirred for 20 min. Cs₂CO₃ (5.68 mmol) was added to this solution and stirred for
1 h. Afterwards, P₃N₃Cl₆ (0.32 mmol) was added to the reaction mixture, and it was left stirring at
room temperature for 7 days, until the reaction was complete, monitored by ³¹P-NMR. The reaction
mixture was then centrifuged at 10,000 rpm for 20 min to remove inorganic salts, the supernatant was
recovered and the solvent was evaporated under reduced pressure. The crude product contained
fully functionalized P₃N₃-[O-C₆H₄-O-(CH₂)ₙ-alkyne]₆ and the unreacted monoalkynes, which were
separated by column chromatography on silica gel using CH₂Cl₂ as the eluent. Once the fraction
corresponding to the functionalized P₃N₃ was obtained, it was recrystallized from cold isopropyl ether.
P₃N₃-[O-C₆H₄-O-(CH₂)ₙ-alkyne]₆ (n = 3 or 4) were obtained as white solids (0.19 mmol, 59% and
0.12 mmol, 52% for C and D, respectively).

P₃N₃-[O-C₆H₄-O-(CH₂)₃-alkyne]₆. ¹H-NMR (400 MHz, DMSO-d₆, δ ppm): 6.80 (d, J = 9.2 Hz, 12H,
Ha), 6.76 (d, J = 9.2 Hz, 12H, Hb), 4.00 (t, J = 2.5 Hz, 12H, Hc), 2.81 (t, J = 2.41 Hz, 6H, C≡C-H),
2.34 (t, J = 2.41 Hz, 12H, He), 1.90 (t, J = 2.41 Hz, 12H, Hd); ¹³C-DEPTQ NMR (101 MHz, DMSO-d₆,


3.2.3. Synthesis of 6-\textsuperscript{O}-monotosyl-\(\beta\)-cyclodextrin (mOTs-\(\beta\)CD) E

Following the procedure previously reported [29], in a round-bottom flask, \(p\)-toluenesulfonic acid (0.0101 mol) and \(p\)-toluenesulfonyl chloride (0.039 mol) were dissolved in 50 mL of \(\text{CH}_2\text{Cl}_2\) and stirred at room temperature for 12 h. Then, the reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was recrystallized (3\(\times\)) with cold hexane, and the product (Ts\(O\)) was allowed to dry under vacuum. Afterwards, in a round-bottom flask, \(\beta\)CD (0.0051 mol) and Ts\(O\) (0.0076 mol) were dissolved in 150 mL of \(\text{H}_2\text{O}\). The reaction mixture was stirred for 2 h at room temperature; after this time, a 2.5-M aqueous NaOH solution was added. The reaction mixture was stirred for 10 min. Subsequently, the mixture was filtered, and the pH of the filtrate was adjusted to 8 with a saturated solution of ammonium chloride to give a precipitate. The mixture was filtered, and the precipitate was recrystallized (3\(\times\)) in acetone. The product E was obtained as a white solid (0.0021 mol, 42%).

\(1^1\text{H-NMR (400 MHz, DMSO-}\delta_{6}\delta \text{ ppm): 7.75 (b, 2H) a, 7.44 (b, 2H) b, 5.83 (d, } J = 6.4 \text{ Hz, 1H) OH2', 5.78 (b, 6H) OH2, 5.71 (b, 7H) OH3, 4.84 (d, } J = 3.9 \text{ Hz, 6H) H1, 4.76 (d, } J = 3.9 \text{ Hz, 1H) H1', 4.50 (m, 6H) OH6, 4.35 (m, 2H) H6'ab, 4.19 (m, 1H) H5', 3.65 (m, 12H) H6ab, 3.60 (b, 7H) H3, 3.51 (m, 7H) H5, 3.30 (m, 7H) H2, 3.22 (m, 7H) H4, 2.42 (d, 3H) c. \(1^3\text{C-NMR (100 MHz, DMSO-}\delta_{6}\delta \text{ ppm): 145.25, 133.09, 130.29, 128.03, 102.39, 101.74, 81.95, 81.21, 73.43, 73.16, 72.84, 72.48, 70.17, 69.36, 60.28, 21.62. MALDI-TOF-MS (m/z): 1311.591 (M + Na)^{+}\).}

3.2.4. Synthesis of 6-\textsuperscript{O}-monoazido-\(\beta\)-cyclodextrin (mN\(3\)-\(\beta\)CD) F

Following a previously reported procedure [32], in a round-bottom flask, mOTs-\(\beta\)CD (0.0016 mol), Na\(\text{N}_3\) (0.005 mol) and KI (0.0008 mol) were dissolved in 8 mL of anhydrous DMF. The reaction mixture was stirred at room temperature for 12 h. After this time, DMF was evaporated under reduced pressure, and the residue was recrystallized in a mixture, \(\text{H}_2\text{O}:\text{Acetonitrile (1:1)}, and allowed to dry under vacuum. The product F was obtained as a white solid (0.0014 mol, 88%).

\(1^1\text{H-NMR (400 MHz, DMSO-}\delta_{6}\delta \text{ ppm): 5.74 (m, 7H) OH2, 5.67 (m, 6H) OH3, 5.62 (d, } J = 2.4 \text{ Hz, 1H) OH3', 4.88 (d, } J = 3.5 \text{ Hz, 1H) H1', 4.83 (m, 6H) OH6, 3.77 (m, 2H) H6', 3.68 (m, 12H) H6, 3.60 (m, 7H) H3, 3.55 (m, 7H) H5, 3.39 (m, 7H) H4, 3.29 (m, 7H) H2. \(1^3\text{C-NMR (100 MHz, DMSO-}\delta_{6}\delta \text{ ppm): 102.38, 102.04, 83.41, 81.99, 73.50, 73.30, 72.85, 72.67, 72.46, 70.63, 60.30, 51.53. MALDI-TOF-MS (m/z): 1182.764 (M + Na)^{+}\).}

3.2.5. Synthesis of \(P_\text{G}_3N\(3\)-[\(O\text{-C}_6\text{H}_4\text{-O-(CH}_2\text{)n-\beta\text{CD}]_8\text{) PDCs (n = 3 or 4) G and H}\)

\(P_\text{G}_3N\(3\)-[\(O\text{-C}_6\text{H}_4\text{-O-(CH}_2\text{)n-alkyne}]) (n = 3 or 4) (0.055 mmol) and mN\(3\)-\(\beta\)CD (0.496 mmol) were dissolved in DMSO (5 mL); this mixture was degassed by bubbling argon for 10 min. A solution of CuSO\(_4\)•5\(\text{H}_2\text{O} (0.055 mmol) in a DMSO:\text{H}_2\text{O mixture (0.5:0.5 mL), followed by a solution of H}_2\text{Asc (0.165 mmol) in DMSO:\text{H}_2\text{O mixture (0.5:0.5 mL), were added dropwise over 5 min. The reaction mixture was heated to } 80 \text{ °C with vigorous stirring and under the argon atmosphere for 7 days, until the reaction was complete, monitored by } ^{31}\text{P NMR. At the end of this time, the reaction mixture was cooled and precipitated dropwise into cold acetone (200 mL). The precipitate was filtered under vacuum.}
The obtained solid was purified by size exclusion chromatography using Bio-Gel® P-10 medium and water as the eluent. Once the corresponding fractions were obtained, they were lyophilized to evaporate the water. PDCs G and H were obtained as white solids (0.041 mmol, 61% and 0.048 mmol, 88% for G and H, respectively).

\[ P_3N_3\{O-C_6H_4-O-(CH_2)_3\}βCD\_6 \]

1H-NMR (400 MHz, DMSO-\(d_6\), \(δ\) ppm): 7.82 (s, 6H, H-triazole), 6.87 (s, 24H, H-a, b), 5.87 (m, 7H, OH-2'), 5.73 (s, 36H, OH-2), 5.70–5.56 (m, 42H, OH-3,3'), 5.05 (s, 7H, H-1'), 4.84–4.78 (m, 42H, H-1; H-6'), 4.56–4.50 (m, 36H, H-6'; OH-6); 4.33 (s, 6H, OH-6”), 4.02 (m, 18H, H-c; H-5'), 3.65–3.59 (m, 138H, H-6, H-3,3', H-5), 3.41–3.28 (m, 84 H, H4,4', H-2,2' overlapped with H2O), 3.12 (m, 7H, H-6”), 2.94 (m, 7H, H-6”), 2.77 (m, 12H, H-e), 2.05 (m, 12H, H-d), 13C-DEPTQ NMR (101 MHz, DMSO-\(d_6\), \(δ\) ppm): 156.55, 144.37, 123.37, 122.15, 115.93, 102.98, 102.02, 84.28, 82.56, 73.05, 72.88, 70.72, 68.13, 60.90, 59.80, 50.37, 29.42, 22.51. 31P-NMR (162 MHz, DMSO-\(d_6\), \(δ\) ppm): s, 9.42. MALDI-TOF-MS: 8254.30 m/z (M + K)+.

\[ P_3N_3\{O-C_6H_4-O-(CH_2)_4\}βCD\_6 \]

1H-NMR (400 MHz, DMSO-\(d_6\), \(δ\) ppm): 7.78 (s, 6H, H-triazole), 6.83 (s, 24H, H-a, b), 5.88 (m, 7H, OH-2'), 5.78–5.72 (s, 36H, OH-2), 5.70–5.63 (m, 42H, OH-3,3'), 5.05 (s, 7H, H-1'), 4.84–4.78 (m, 42H, H-1; H-6'), 4.58–4.49 (m, 36H, H-6'; OH-6); 4.34 (s, 6H, OH-6”), 3.96 (m, 18H, H-c; H-5'), 3.65–3.59 (m, 138H, H-6, H-3,3', H-5), 3.41–3.38 (m, 84 H, H4,4', H-2,2' overlapped with H2O), 3.12 (m, 7H, H-6”), 2.94 (m, 7H, H-6”), 2.77 (m, 12H, H-e), 1.76 (m, 24H, H-d, e); 13C-DEPTQ NMR (101 MHz, DMSO-\(d_6\), \(δ\) ppm): 156.56, 147.25, 123.86, 122.86, 122.11, 115.96, 102.98, 101.98, 84.18, 82.80, 82.33, 81.62, 73.94, 72.91, 68.38, 60.73, 59.77, 50.79, 29.27, 26.29, 22.50. 31P-NMR (162 MHz, DMSO-\(d_6\), \(δ\) ppm): s, 9.42. MALDI-TOF-MS: 8254.30 m/z (M + K)+.

### 3.3. Characterization

1H, 13C and 31P-NMR measurements were carried out on a Bruker spectrometer (Bruker, Beerlika, MA, USA) (400 MHz) using DMSO-\(d_6\) as solvent. Chemical shifts are reported in ppm (\(δ\)), and the signals were described as singlet (s), doublet (d), triplet (t) and multiplet (m); coupling constants (\(J\)) were reported in Hz. DART and MALDI-TOF mass measurements were performed on a JEOL JMS-AX505-HA instrument (Peabody, MA, USA) and on a Bruker Daltonics Flex Analysis instrument (Bruker, Beerlika, MA, USA). Matrices of 2,5-dihydroxybenzoic acid (DHB), dithranol (DIT) and 2-acetylphloroglucinol (THAP) were used for MALDI-TOF.

### 3.4. Determination of Water Solubility for \(P_3N_3\{O-C_6H_4-O-(CH_2)_3\}βCD\_6\) PDCs

The determination of the solubility of the \(P_3N_3\{O-C_6H_4-O-(CH_2)_3\}βCD\_6\) PDCs was carried out according to a method previously reported by Jozwiakowski and Connors [36], with modifications. One gram of the compound was placed in three amber vials of 5 mL with a screw cap, and 1 mL of water was added. The vials were sealed with parafilm to avoid water evaporation. They were stirred in an oil bath at a constant temperature of 25 ± 0.01 °C for 48 h. The supernatant was separated from the solid phase by filtration through a Milli-Q membrane (pore size of 0.45 μm) by injection of the mixture into disposable plastic syringes (Franklin Lakes, NJ, USA) of 3 mL at 25 °C. The supernatant of each sample was placed in three different vials. The samples were lyophilized for 48 h, and the obtained solids were weighed on a scale with an uncertainty of ±0.0001 g.

### 4. Conclusions

In this work, two novel phosphorus dendritic compounds (PDCs) containing a \(P_3N_3\) as the core and βCD units as terminal groups were designed. The synthesis was carried out using the CuAAC reaction, giving high yields and products that were purified by a simple method. The complete functionalization of the \(P_3N_3\) core of the new molecules was carried out using two aliphatic chains of different lengths as the linker. We found that there was no significant impact of the aliphatic chain length, since, with both linkers, the functionalization was complete. This is the first report for
PDCs of the type $P_3N_3$-[O-C$_6$H$_4$-O-(CH$_2$)$_n$-O-(CH$_2$)$_2$-βCD]$_6$ arising from a novel design of water-soluble PDCs. The potential use of these new dendrimers as drug carriers for biomedical applications is currently under study.

**Supplementary Materials:** The following are available online: NMR & MS spectrum of the compounds.

**Author Contributions:** E.R.; I.G.-M.; K.I.M.-C.C. and A.-M.C.—design of the dendrimers. K.S.-M.—synthesis of the dendrimers. K.S.-M. and I.G.-M.—characterization of the dendrimers. K.S.-M. and I.G.-M.—contributed to manuscript writing. J.I.—data curation. E.R.; M.V.; J.I.; K.I.M.-C.C. and A.-M.C.—edited and revised the manuscript. E.R.—supervision. All authors have read and agreed to the published version of the manuscript.

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**Sample Availability:** Samples of the compounds are not available from the authors.

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