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Controlling Multivalency and Multimodality: Up to Pentamodal Dendritic Platforms Based on Diethylenetriaminepentaacetic Acid Cores

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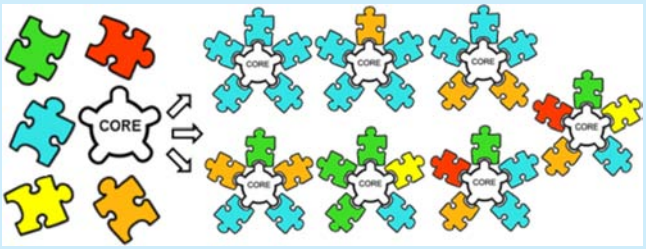
Supporting Information

ABSTRACT: A highly versatile synthetic strategy is described to generate multimodal and multivalent platforms based on a diethylenetriaminepentaacetic (DTPA) core. Compounds with different functionalization patterns, from mono- to pentamodal, have been prepared using robust and simple chemistry.



State-of-the-art techniques used in chemistry, biology, and transversal disciplines such as nanotechnologies have contributed to the advancement of biomedical research, yielding the development of innovative strategies for therapy,[1] diagnostics,[2] or a combination of the two, namely, theranostics.[3] To make further progress, new methodologies to facilitate combination therapies,[4] improved systems to deliver drugs at a precise tissue or organ, and better imaging agents,[5] as well as new multifunctional molecules designed to study complicated biological processes, are required. In this regard, the generation of new well-defined multimodal molecules that allow the incorporation of functionalities such as drugs, imaging agents, targeting molecules, or a combination of the same, onto a single scaffold, in a precise and controlled manner is particularly relevant. From a chemical point of view, these types of platforms are considered a synthetic challenge or, as mentioned elsewhere, a synthetic "tour-de-force".[6]

Our laboratory has spent recent years working on the preparation of dendritic-type structures based on oligoethylene glycol (OEG) moieties. Dendrimers have become attractive scaffolds in nanomedicine;[7] however, their usefulness in future biomedical applications demands well-defined monodisperse compounds whose surfaces can be modified with different molecules in controlled ratios, thus avoiding statistical modifications that would give rise to undesired polydisperse materials.[6] To date, few examples of these new generation dendritic structures have been described, these mainly with two differentiated functionalities,[8] some with three,[9] and very few with more than three.[10] Their syntheses rely on several strategies: (1) appropriate combination of diverse functional groups;[8b,9a] (2) incorporation of predifferentiated bifunctional molecules (e.g., amino acids)[9b,d] or building blocks that are easily desymmetrized;[8c,f] (3) linkage of two distinct dendritic wedges to generate Janus dendrimers, mainly taking advantage of the emergence of chemoselective ligations;[8d,e] and (4) convergent synthesis based on the control of the reactivity of a particular core unit.[8a,9c,10a,b] Most of these approaches focus on the introduction of diversity at late stages of the synthesis. In contrast, our method is more oriented to control the differentiation from the very beginning, at the core level. Diethylenetriaminepentaacetic acid (DTPA) is a molecule used traditionally as a chelating agent.[11] It contains five carboxylic acids and can serve as a source of multivalency and, with proper modification, also of multimodality. Here we describe the synthesis of these versatile compounds derived from DTPA and their use for the preparation of monodisperse oligoethylene glycol first-generation dendritic platforms, as representative models of the multimodal and multivalent platforms that can be generated with this strategy. Several functionalities varying from 1 to 5 can be incorporated in different ratios, giving rise to up to pentamodal structures (Figure 1). These products can be considered not only as scaffolds to display diverse functionalities in a single molecule but also as pieces to create higher generation dendrimers[12] and can also be used to introduce controlled biofunctionality in other molecular systems (e.g., hydrogels, biomaterial surfaces, proteins, antibodies, nanoparticles).

Three DTPA derivatives, **8**, **9**, and **10** (Scheme 1), were prepared as cores, covering all possibilities in terms of multimodality (Figure 1). Compound **8** (DTPA bisanhydride)

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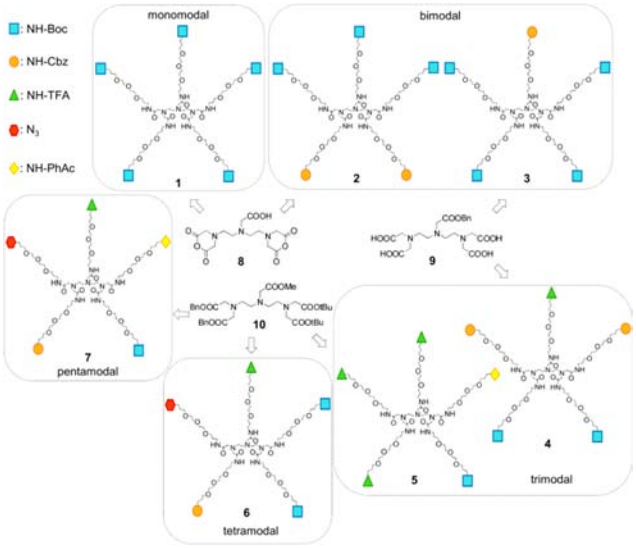


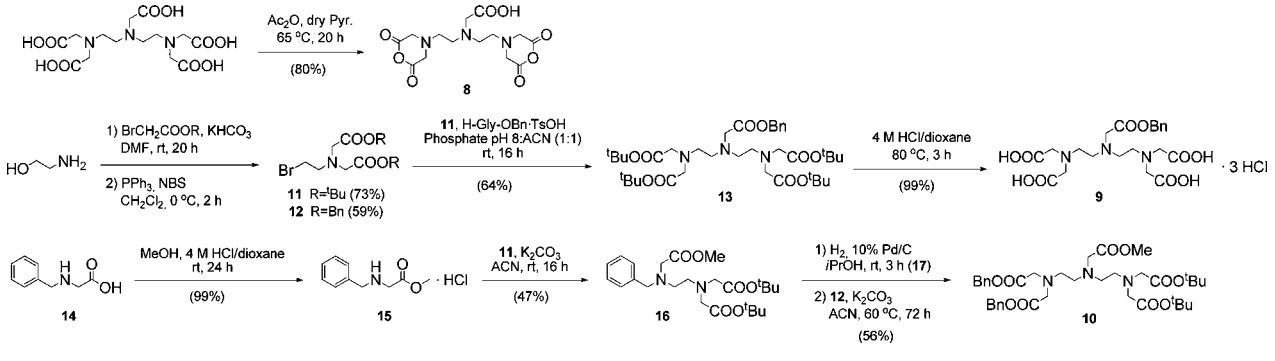
Figure 1. Multivalent and multimodal dendritic platforms **1**−**7** based on DTPA core and OEG branches.

was obtained easily in a single-step synthesis starting from commercial DTPA by reaction with a mixture of Ac_2O and pyridine at 65 °C.[13,14] Compound **9** was prepared following a four-step synthesis, previously described by our group.[12] Briefly, ethanolamine was dialkylated with *tert*-butyl bromoacetate, then the hydroxyl group was replaced by bromine, and this bromo derivative **11** was used to dialkylate glycine benzyl ester. *tert*-Butyl ester groups of **13** were removed by treatment with acid (HCl). The overall yield of the synthesis was 46%. Finally, trimodal scaffold **10** was prepared as follows: *N*-benzylglycine (**14**) was converted to the corresponding methyl ester by treatment with methanol under acidic catalysis. The resulting hydrochloride salt **15** was alkylated with 1 equiv of bromo derivative **11** under mild basic conditions to afford **16**. After removal of the benzyl group by Pd/C-catalyzed hydrogenolysis, compound **17**[15] was alkylated with bromo derivative **12**. This four-step synthesis was performed in low-gram scale (1−2 g) with an overall yield of 26%. Using these three core compounds, we successfully prepared **1**−**7** (Figure 1). Here we used short and monodisperse diamino oligoethylene glycol (OEG) branches to prepare the dendritic compounds. Diversity was achieved using a different set of compatible protecting groups to mask one amino function of the OEG moieties: Boc (removed by treatment with acid); Cbz (removed by hydrogenation); trifluoroacetylamide (removed by basic treatment); PhAc[16] (removed by penicillin G acylase); and N_3 (reduced to amine with phosphines or to be used directly in click chemistry[17]). The corresponding protected OEGs were prepared from commercially available Boc-OEG (1-(*tert*-butoxycarbonyl-amino)-4,7,10-trioxa-13-tridecanamine).

One of the strong points of the synthesis lies in the robustness of the chemical reactions used, which consist mainly of the removal of protecting groups and the formation of amide bonds, either by acylation using the phosphonium salt PyBOP as the coupling reagent[18,19] or by preparing cyclic anhydrides to activate the corresponding carboxylic acids (Scheme 2). We achieved clean reaction crude products and were able to isolate the desired compounds using simple workup procedures based on aqueous (basic and/or acidic) extractions and precipitation protocols, thereby avoiding more tedious chromatographic purifications.

The simplest compound containing five equivalent positions was prepared by the coupling of five branches of Boc-OEG to core **8** using PyBOP as activating agent. Compound **1** was obtained with 96% yield. The differentiation of two positions was designed with two distinct ratios, the 3−2 (**2**) and the 4−1 (**3**). In the former, DTPA bisanhydride (**8**) was reacted with Cbz-OEG to incorporate two OEG chains (**23**). Afterward, the remaining three carboxylic acids were acylated with Boc-OEG using PyBOP as activating agent to afford **2** with an overall yield of 74% (2 steps). The preparation of the 4−1 version (**3**) was achieved by incorporation of a Boc-OEG moiety to each of the four carboxylic acids of **9**, mediated by the action of PyBOP. The benzyl group of **24** was then removed by catalytic hydrogenation, and the resulting carboxylic acid was derivatized to the amide with Cbz-OEG and PyBOP, furnishing compound **3** with 70% yield (3 steps). Once the simplest platforms had been prepared, the use of protecting groups, selective elimination, and amide formation was not enough to gain additional differentiation capacity. It was then necessary to distinguish between free carboxylic acids once they were deprotected. This was accomplished taking advantage of the favored formation of six-membered ring cyclic anhydrides between pairs of carboxylic acids in a DTPA-like molecule. These cyclic anhydrides were easily desymmetrized by reaction with only 1 equiv of amino-OEG to form the amide, rendering a free carboxylic acid that could be acylated in a further step using an orthogonally protected amino-OEG and PyBOP. Combining the use of protecting groups, cyclic anhydride formation, and subsequent desymmetrization, scaffolds **9** and **10** were precursors of two platforms with three differentiated positions, 2−2−1 (**4**) and 3−1−1 (**5**), respectively. In the first case, core **9** was treated with Ac_2O in pyridine at 65 °C, and the corresponding bisanhydride was formed. This derivative reacted

Scheme 1. Synthesis of Cores 8, 9, and 10



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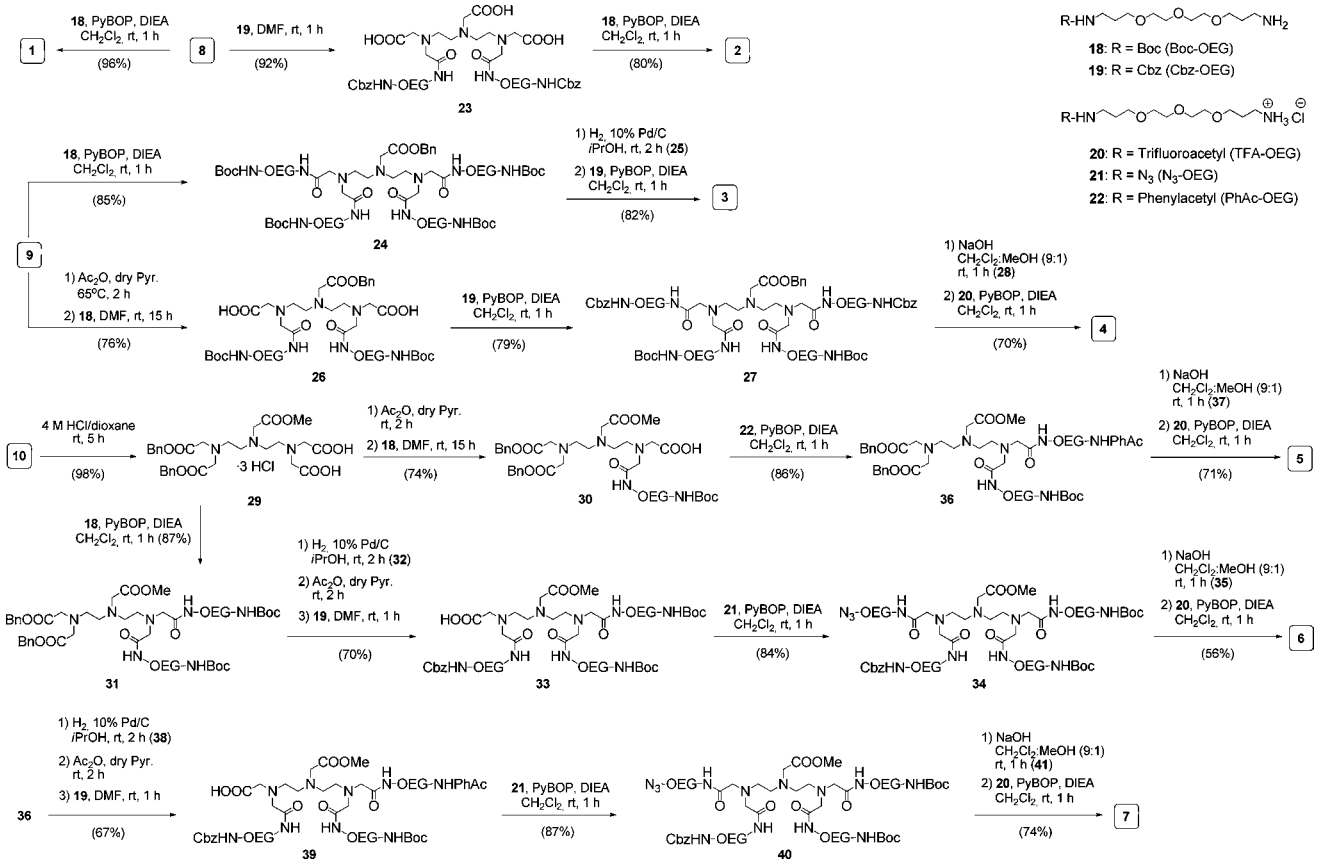
Scheme 2. Synthesis of Dendritic Platforms 1−7



with Boc-OEG, and two chains were incorporated to form **26**. The free carboxylic acids were then transformed into the corresponding amides performing the coupling reaction with Cbz-OEG to obtain **27**. Finally, benzyl ester was removed by treatment with NaOH in CH_2Cl_2/MeOH,[20] and the derivative TFA-OEG was used to form the amide bond with the resulting carboxylic acid, affording **4** with an overall yield of 42% (5 steps). For the 3−1−1 version (**5**), the starting point was the core **10**. *tert*-Butyl groups were removed by treatment with acid (HCl), and the two carboxylic acids were desymmetrized, first by formation of the cyclic anhydride and subsequent opening by Boc-OEG (**30**), followed by acylation with PhAc-OEG (**36**). Benzyl and methyl esters were removed by basic treatment with NaOH in CH_2Cl_2/MeOH, and the TFA-OEG branches were introduced in the three carboxylic acids formed. This route rendered compound **5** with 44% yield (6 steps). Core **10** was also used to prepare the tetramodal platform 2−1−1−1 (**6**). As in the previous example, *tert*-butyl groups were removed; however, in this case, both carboxylic acids were derivatized to amides with Boc-OEG to obtain **31**. Benzyl esters were removed by catalytic hydrogenation, and the desymmetrization via cyclic anhydride was performed using Cbz-OEG first (**33**) and N_3-OEG later to obtain compound **34**. Finally, methyl ester was removed by basic treatment with NaOH in CH_2Cl_2/MeOH, and the carboxylic acid was acylated with TFA-OEG to complete the synthesis of **6** with a 28% overall yield (8 steps). Finally, the highest degree of multimodality was reached in the platform 1−1−1−1−1 (**7**). After conversion of trimodal core **10** into **36**, benzyl esters were removed by catalytic hydrogenation. A second desymmetrization via cyclic anhydride was achieved using Cbz-OEG in the first step (**39**) followed by N_3-OEG coupling (**40**). Finally, methyl ester was removed with NaOH in CH_2Cl_2/MeOH, and TFA-OEG was incorporated, furnishing the pentamodal platform **7** (27% yield, 10 steps).

In this set of multimodal platforms, amines or masked amines were selected as functional groups. Nonetheless, other functional groups, such as carboxylic acids, alcohols, or thiols, among others, can also be introduced easily into these synthetic protocols. The platforms described comprised OEG chains; however, it is important to highlight that the versatility of the cores and the synthetic procedures shown here go beyond the scope of short OEGs. Thus, many other "puzzle pieces" of distinct natures could be used to build these multimodal platforms, depending on the final applications. Several examples containing peptides, different length OEGs, fluorophores, or aliphatic chains are described in Supporting Information (compounds **42** (3−2 platform), **43** (2−2−1 platform), and **44** and **45** (4−1 platforms)).

In conclusion, here we prepared a new class of multimodal platforms based on a DTPA core. These platforms were functionalizable "a la carte", ranging from mono- to pentamodal patterns. The compounds were obtained using robust and scalable synthetic protocols in combination with a set of compatible protecting groups, and avoiding tedious purification steps. With this methodology complicated chemical structures become accessible by using simple chemical reactions.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures; spectroscopic and analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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