ORGANIC LETTERS

2006
Vol. 8, No. 20
4421−4424

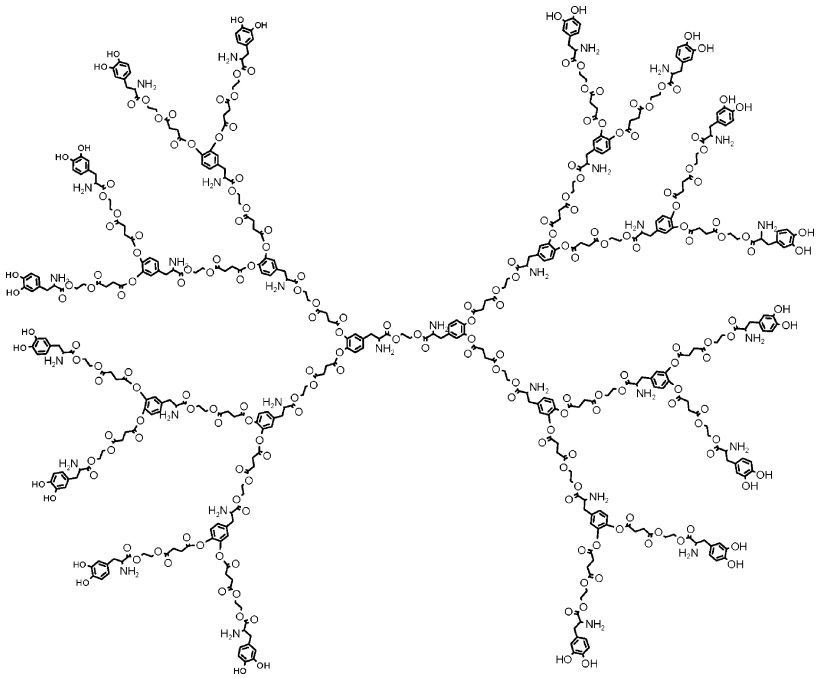
Synthesis and Characterization of Water-Soluble and Photostable L-DOPA Dendrimers

Shengzhuang Tang, Lynda J. Martinez, Ajit Sharma, and Minghui Chai*

Department of Chemistry, Central Michigan University, Mt. Pleasant, Michigan 48859
minghui.chai@cmich.edu

Received June 13, 2006

ABSTRACT



A small drug molecule, L-DOPA, was converted into well-defined dendritic macromolecules. Their monodisperse nature was shown by NMR, MALDI-TOF-MS, and PAGE. A third-generation L-Dopa dendrimer contained 30 L-Dopa residues, which made up its core, branches, and periphery. Individual L-Dopa moieties in the dendrimer were connected to one another via hydrolyzable diester linkages. These Dopa dendrimers showed a 20-fold increase in aqueous solubility and enhanced photostability in solutions over L-Dopa under identical conditions.

L-DOPA (levodopa, 3,4-dihydroxy-L-phenylalanine) is a drug used to treat Parkinson's disease. It is a prodrug capable of passing the blood−brain barrier. It is decarboxylated in the brain to become dopamine, the neurotransmitter, by the enzyme aromatic-L-amino acid decarboxylase. L-DOPA can relieve some of the dopamine deficit seen in Parkinsonism.[1] However, it also induces side effects such as dystonia and dyskinesia after large doses or chronic use. Slow release of L-DOPA (e.g., long-acting forms of Sinemet and Madopar) has shown the reduction of the problems associated with long-term therapy.

Dendrimers are promising nanomaterials for medical and pharmaceutical applications because they may improve the therapeutic efficacy of many low MW drugs by utilizing the dendritic voids inside to physically encapsulate the drugs or the terminal groups on the periphery to chemically conjugate them.[2] To achieve high drug loading, dendrimers of relatively large size have to be used. Linear polymer drugs such as

(1) *The Columbia Electronic Encyclopedia*, 6th ed.; Columbia University Press: New York, 2005.

10.1021/ol061449l CCC: $33.50
Published on Web 09/08/2006

PolyAspirin truly offer a huge capacity of drugs and an easily manipulated system for drug delivery.[3] However, the release of drug units in a linear polymeric drug can still be "sudden" because the hydrolytic degradation may occur at any spot of the polymer backbone or even at multiple spots simultaneously.

In this communication, we demonstrate a novel approach to incorporate multiple drug units (i.e., L-DOPA) into a cascade structure to form a dendrimer prodrug (Figure 1)

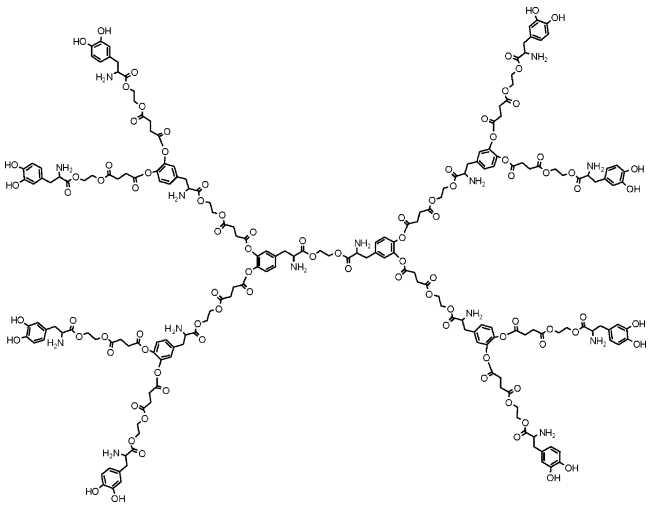
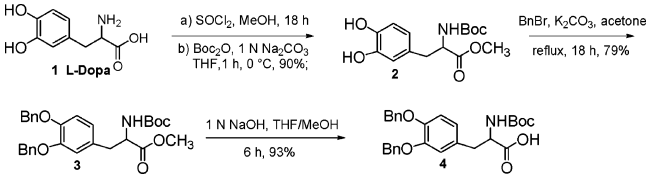


Figure 1. Structure of the second-generation L-DOPA dendrimer prodrug (HO-G2-NH_2).

based on L-DOPA.[4] This is believed to be the first time that a dendrimer is made of and used as an intrinsic drug or prodrug instead of as a carrier for encapsulation or conjugation. Such a well-defined dendritic architecture will be expected to provide a sequential and better controlled release profile by losing the periphery drug entities first, then the interior ones, and lastly the core units.

Synthesis of the L-DOPA dendrimer prodrugs started from commercially available L-DOPA **1** (Scheme 1), which was

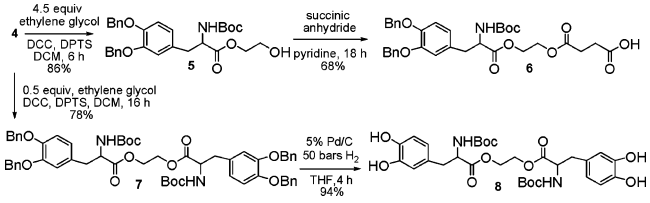
Scheme 1. Synthesis of Boc- and Bn-Protected L-DOPA-COOH[4]



converted to the corresponding methyl ester and then immediately N-protected by treatment with di-*tert*-butyldicarbonate to synthesize HO-DOPA-NHBoc-COOMe **2**.[5] Then, the catechol function of **2** was treated with potassium carbonate, sodium iodide, and an excess of benzyl bromide in refluxing acetone to give the BnO-DOPA-NHBoc-COOMe **3**,[6] which was hydrolyzed by 1 N aqueous NaOH in MeOH/THF at room temperature followed by acidification to afford BnO-DOPA-NHBoc-COOH **4**.[7]

Scheme 2 shows the synthesis of the building block **6** and the Boc-protected core **8**. Compound **4** was treated with 4.5 equiv of ethylene glycol in the presence of dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)-pyridinium *p*-toluenesulfonate (DPTS) to give **5**,[8] which was then stirred with succinic anhydride in pyridine to provide the building block **6** for the continuous synthesis of high-generation dendrimer prodrugs.[9] The BnO-G0-NHBoc **7** was produced by simultaneously coupling two units of **4** with ethylene glycol in the presence of DCC and DPTS and then was hydrogenolyzed with a palladium catalyst (5% Pd/C) to yield the HO-G0-NHBoc **8**,[10] which was used as the core to develop high-generation dendrimer prodrugs.

Scheme 2. Synthesis of Building Block **6** and Core **8** (Boc-G0)



The synthesis of a high generation of drug dendrimers was achieved with a routine coupling and deprotecting procedure (Scheme 3). The core **8** was coupled with four units of the building block **6** to afford BnO-G1-NHBoc **9**, which was then deprotected back to the catechol function by hydrogenolysis with H_2/Pd−C (Parr apparatus) to give HO-G1-NHBoc **10**. The deprotection of amine groups of **10** via acidification gave the desired first-generation L-DOPA

(2) (a) Pasut, G.; Scaramuzza, S.; Schiavon, O.; Mendich, R.; Veronese, F. M. *J. Bioact. Compat. Polym.* **2005**, *20*, 213−230. (b) Bryan, J. *Pharma. J.* **2004**, *273*, 793−794. (c) Delort, E.; Darbre, T.; Reymond, J.-L. *J. Am. Chem. Soc.* **2004**, *126*, 15642−15643. (d) Liu, M.; Kono, K.; Fréchet, J. M. J. *J. Polym. Sci. Part A: Polym. Chem.* **1999**, *37*, 3492−3503.

(3) (a) Whitaker-Brothers, K.; Uhrich, K. E. *J. Biomed. Mater. Res.: Part A* **2006**, *764*, 470−479. (b) Miao, J.; Xu, G.; Zhu, L.; Tian, L.; Uhrich, K. E.; Aliva-Orta, C. A.; Hsiao, B. S.; Utz, M. *Macromolecules* **2005**, *38*, 7074−7082. (c) Prudencio, A.; Schmeltzer, R. C.; Uhrich, K. E. *Macromolecules* **2005**, *38*, 6895−6901. (d) Harten, R. D.; Svach, D. J.; Schmeltzer, R.; Uhrich, K. E. *J. Biomed. Mater. Res.: Part A.* **2005**, *72A*(4), 354−362. (e) Schmeltzer, R.; Schmalenberg, K.; Uhrich, K. E. *Biomacromolecules* **2005**, *6*(1) 359−367.

(4) Chai, M.; Tang, S. Method of Preparing Dendritic Drugs. U.S. Provisional Patent Application, January 24, 2006.

(5) (a) Hunter, L.; Hutton, C. A. *Aust. J. Chem.* **2003**, *56*, 1095−1098. (b) Gaucher, A.; Barbeau, O.; Hamchaoui, W.; Vandrommer, L.; Wright, K.; Wakselman, M.; Mazaleyrat, J. P. *Tetrahedron Lett.* **2002**, 8241−8244.

(6) Clary, M. T.; Adejare, A.; Shams, G.; Feller, D. R.; Miller, D. D. *J. Med. Chem.* **1987**, *30*, 86−90.

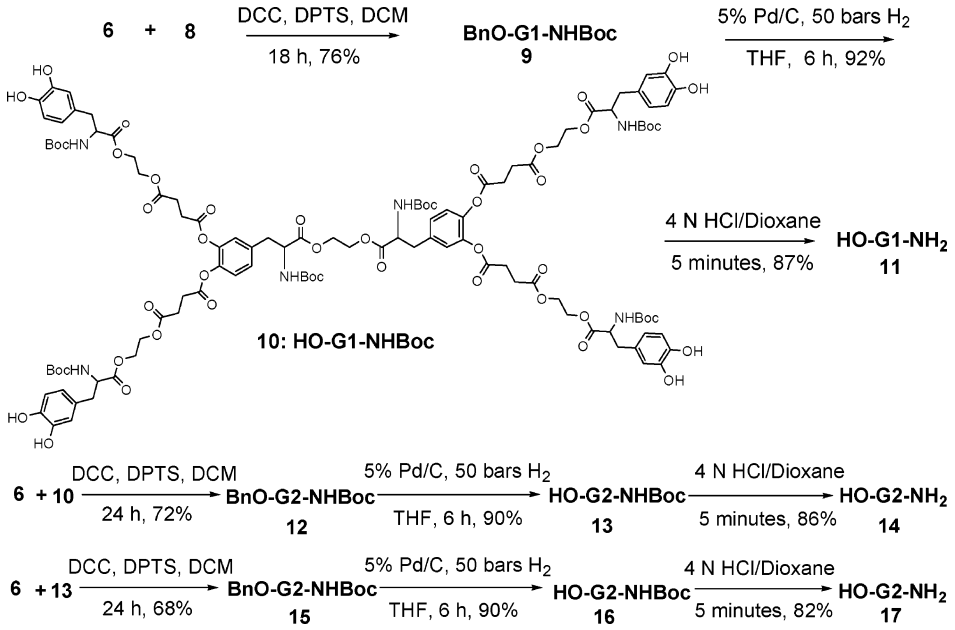
(7) Hu, B. H.; Messersmith, P. B. *Tetrahedron Lett.* **2000**, *40*, 5795−5798.

(8) Moore, J. S.; Stupp, S. I. *Macromolecules* **1990**, *23*, 65−70.

(9) Luman, N. R.; Smeds, K. A.; Grinstaff, M. W. *Chem.−Eur. J.* **2003**, *9*, 5618−5626.

(10) Hanessian, S.; Liak, T. S.; Vanasse, B. *Synthesis* **1981**, *5*, 396−397.

Scheme 3. Syntheses of Dendritic Prodrugs G1−G3



dendrimer prodrug (HO-G1-NH_2) **11**, which was characterized by NMR and MALDI-TOF-MS (observed $[M + Na]^+$ 1735.7 Da and $[M + K]^+$ 1751.7 Da; $C_{80}H_{92}N_6O_{36}$ requires 1713.6 Da). Meanwhile, HO-G1-NHBoc **10** was coupled with eight units of building block **6** to yield BnO-G2-NHBoc **12**, which was hydrogenolyzed to generate HO-G2-NHBoc **13**. Boc deprotection by acidifying **13** then afforded the desired second-generation L-DOPA dendrimer prodrug (HO-G2-NH_2) **14**, which was also characterized by NMR and MALDI-TOF-MS (observed $[M + Na]^+$ 4324.2 Da and $[M + K]^+$ 4339.8 Da, $C_{200}H_{228}N_{14}O_{92}$ requires 4300.2 Da). Furthermore, coupling HO-G2-NHBoc **13** with 16 units of the building block **6** afforded the fully protected third generation of dendrimer prodrugs BnO-G3-NHBoc **15**, which was then converted into the third generation of the dendritic L-DOPA prodrug, HO-G3-NH_2 **17**, via the same deprotection steps as those shown in the previous syntheses of the dendrimers **11** and **14**. NMR and MALDI-TOF-MS were also used for the characterization of **17** (observed $[M + H]^+$ 9474.0 Da, $C_{440}H_{500}N_{30}O_{204}$ requires 9472.8 Da).

Gel electrophoresis on 10% acrylamide gels was performed on these newly synthesized dendrimers (G0−G3) under acidic conditions as reported earlier.[11] The electropherogram shown in Figure 2 clearly showed relatively sharp and discrete bands representing species with low polydispersity. For comparison, the commercially available ethylenediamine−core amine terminated G4 PAMAM [generation 4 poly(amido amine)] dendrimer was run under the same conditions. As with the PAMAM dendrimers, our dendritic L-DOPA prodrugs showed a decrease in migration with increasing generation number. The HPLC chromatograms of G0−G3 L-DOPA dendrimers[12] showed that the clean products were obtained from the syntheses.

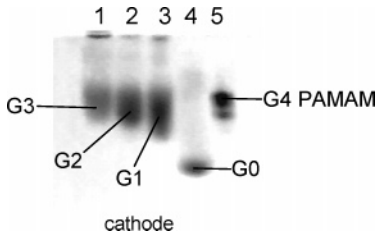


Figure 2. PAGE of novel dendritic L-DOPA prodrugs (G0−G3). Lane assignments are as follows: 1 = 50 μg of G3; 2 = 50 μg of G2; 3 = 50 μg of G1; 4 = 150 μg of G0; 5 = 5 μg of ethylenediamine−core amine terminated PAMAM dendrimer G4.

Solubility studies on these prodrugs have been performed in deionized water and 140 mM NaCl solution. The results clearly show that the newly synthesized L-DOPA dendrimers (>50 mg/mL) are much more soluble in water than L-DOPA (<5 mg/mL). Photodegradation studies have also been conducted for these dendritic prodrugs in comparison with L-DOPA. The results from UV−vis studies[12] demonstrate that the dendritic prodrugs are much more photostable than L-DOPA. The hydrolysis of these novel dendritic prodrugs was also studied by NMR and HPLC.[12] The results display a sequential degradation mechanism for these dendritic prodrugs.

In conclusion, we have reported a successful synthetic route of novel dendritic L-DOPA prodrugs (generations

(11) Sharma, A.; Mohanty, D. K.; Desai, A.; Ali, R. A. *Electrophoresis* **2003**, *24*, 2733−2739.

(12) The data are available in the Supporting Information.

1−3). More biological and pharmacological studies on these new dendritic prodrugs will be pursued.

Acknowledgment. This research was supported by the U.S. National Science Foundation (NUE-0407298), Research Corporation (CC-6059), American Chemical Society Petroleum Research Fund (#40998-GB4), Army Research Laboratory (ARL), and Central Michigan University. We thank M. Jurcak and S. Dennis (Department of Chemistry, Central Michigan University) for HPLC experiments on the dendrimers.

Supporting Information Available: (1) Detailed experimental procedures, (2) gel electrophoresis measurements, (3) NMR spectra of important intermediates and final products, (4) UV−vis data on the photostability of L-DOPA and G3 L-DOPA dendrimers, (5) HPLC chromatograms of L-DOPA dendrimers, and (6) NMR and HPLC studies on the degradation of L-DOPA dendrimers. This material is available free of charge via the Internet at http://pubs.acs.org.

OL061449L