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Synthesis of a dendritic estrogen cluster: A potential tool for studies of nuclear *versus* extranuclear pathways of estrogen actions

Jian Chen [a], Hu Zheng [b], Yan Song [a], Yu Feng Liang [b], Qing Rong Qi [b,*]

[a] *Functional Food (Drug) Laboratory, Institute of Agro-Product Processing Science and Technology, Sichuan Academy of Agricultural Science, Chengdu 610066, China*

[b] *Key Laboratory of Drug-Targeting and Drug-Delivery Systems of the Ministry of Education, Department of Medicinal Chemistry, West China School of Pharmacy, Sichuan University, Chengdu 610041, China*

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Abstract

A novel estrogen dendrimer has been synthesized through a combination of divergent and convergent approaches in 9 practical steps and in good yields. It was characterized and confirmed by elemental analysis, FT-IR, MS, ^{1}H NMR, ^{13}C NMR. The dendrimer contains 16 estrone units and is potentially a useful tool for the studies of estrogen actions.

Keywords: Estrogen dendrimer; Synthesis; Nuclear and extranuclear; Receptor

Estrogen plays important roles in human physiology and pathology. It acts through two pathways. One is *via* classical nuclear estrogen receptors (ERs), namely ER_{α} and ER_{β}, altering target gene transcription [1]. The other is an extranuclear pathway *via* the modulation of signaling through secondary messengers, more commonly associated with growth factor and G protein-coupled receptors (GPR30) [2,3]. The extranuclear pathway has become an active topic as a novel therapeutic target in estrogen-related diseases [4–6].

To carry out selective studies of nuclear and extranuclear pathways of estrogen actions, one important approach is to create membrane-impermeable estrogen ligands, based on the assumption that they only stimulate receptors with binding sites on the external face of the plasma membrane, without stimulation of nuclear ERs [4].

In this work we report a novel design and synthesis of a dendritic estrogen cluster **11** (DEC, Fig. 1). The DEC compound might be a potential tool for the studies of estrogen activities that follow extranuclear pathways. Compared with previously reported membrane impermeable estrogen macromolecular conjugates [7–10], the new dendritic estrogen cluster overcomes many of their reported defects. For example, DEC contains exactly 16 estrone molecules on the surface of the dendrimer, not ''approximately''. It is a structurally well-defined single unique molecule with a molecular weight 6744.4 ($C_{401}H_{516}O_{88}$), so there is no ''free'' estrogen in the cluster. The second generation dendrimer is composed of neutral aliphatic extension chains with appropriate length. The chains have chemically stable ether and ester bonds, not non-covalent or chemical unstable oxime linkages. There is no amino group in the

* Corresponding author.
E-mail address: qiqingronghh@yahoo.com.cn (Q.R. Qi).

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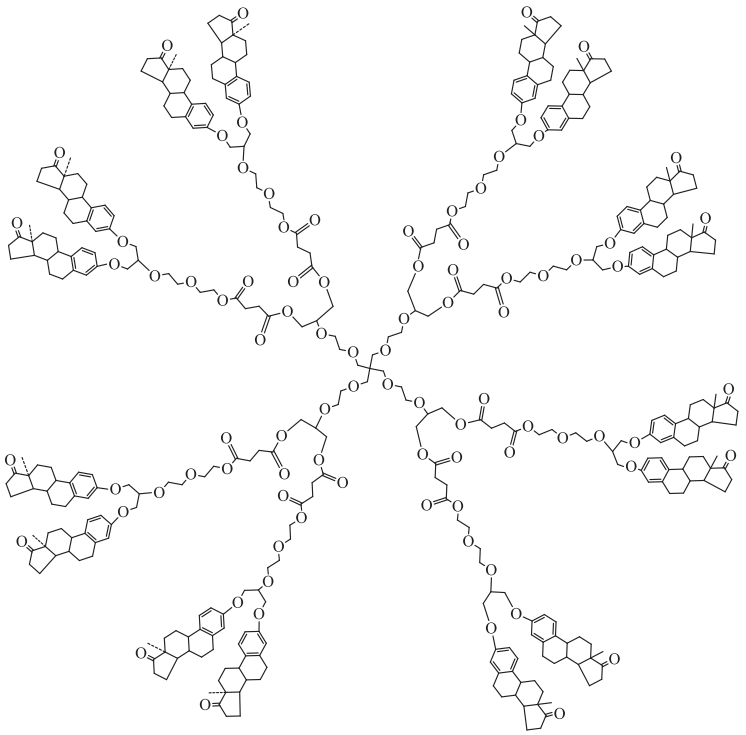


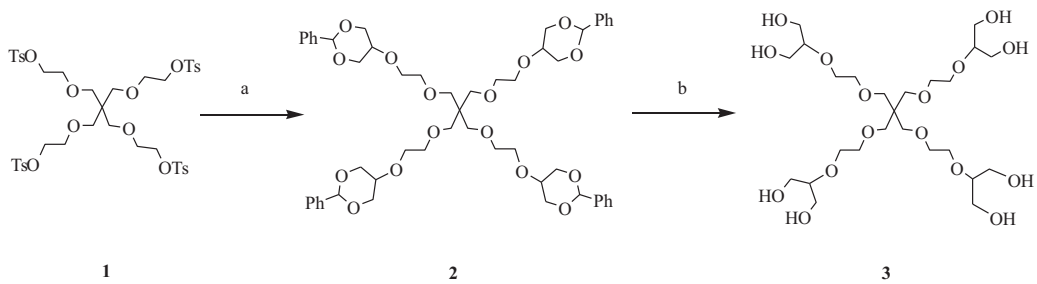
Fig. 1. Chemical structure of the dendrimer **11**.

dendrimer, so it bears no charge at physiological pH values, so there is no disturbance of cell membrane permeability. Furthermore, the aliphatic chain will not introduce physiological side effects, other than estrogen action, before or after the DEC degradation.

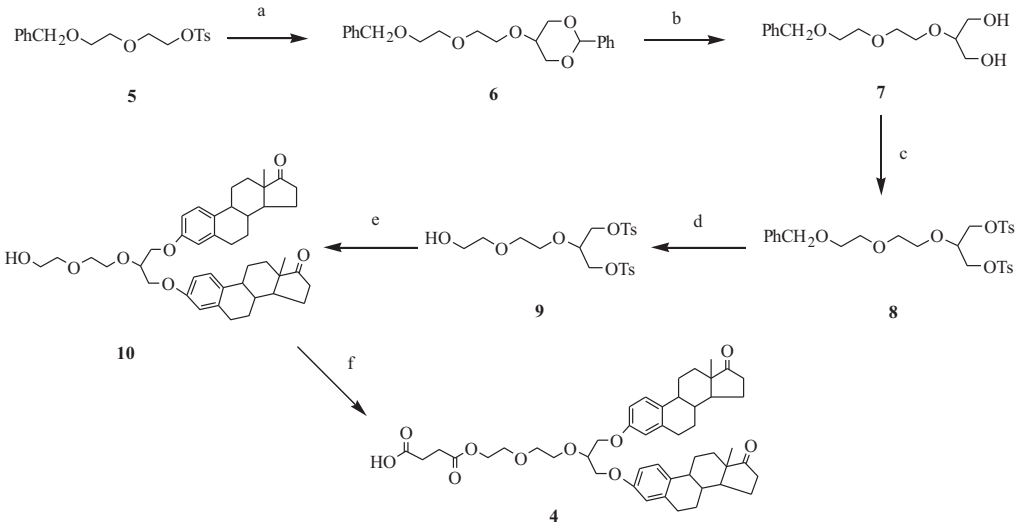
The synthesis of DEC was achieved through a strategy combining divergent and convergent approaches, as shown in Schemes 1–3.

The first generation eight directional multi-hydroxyl aliphatic dendrimer **3** was synthesized using a divergent method, as shown in Scheme 1. The starting compound **1** [11] was reacted with 1,3-*O*-benzylidene glycerol [12] to produce **2**. 1,3-*O*-Benzylidene glycerol has *cis*- and *trans*-forms. The hydroxyl group in the *trans*-form has higher reactivity because of less steric hindrance, so we used *trans*-1,3-*O*-benzylidene glycerol to react with **1** to obtain **2** with 72% yield. The acetal groups in **2** were very sensitive to acid conditions so it had to be handled carefully in the preparation and testing process to avoid a complicated decomposition mixture. On the other hand, **2** was easily hydrolyzed to expose eight hydroxyl groups in the presence of *p*-TsOH in methanol, resulting in the oily compound **3** in 90% yield.

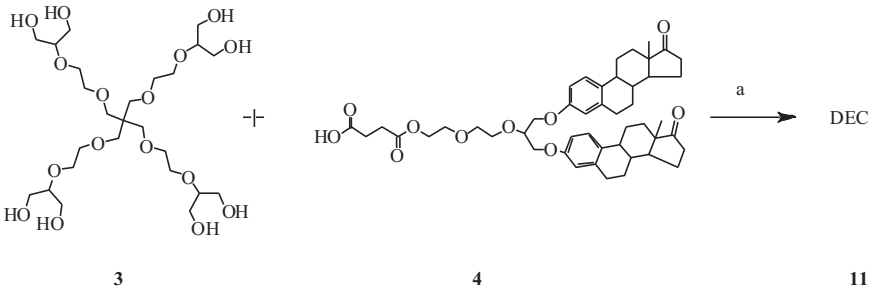
The important branched side chain **4**, which possesses two estrone molecules, was synthesized from the extension chain diethylene glycol derivative **5** [11], as shown in Scheme 2. In a similar way to the preparation method of



Scheme 1. (a) *trans*-1,3-*O*-Benzylidene glycerol (4.4 equiv.)/THF/NaH/reflux 48 h, 72% and (b) *p*-TsOH/MeOH/rt, 6 h, 90%.



Scheme 2. (a, b) *cis*,*trans*-1,3-*O*-Benzylidene glycerol (1 equiv.)/THF/NaH/reflux 3 h; *p*-TsOH/MeOH/rt, 4 h, 70%; (c) *p*-TsCl (2.15 equiv.)/THF/5 mol/L NaOH, 5 °C, 5 h/rt 1 h/99.5%; (d) 10% Pd/C, 1 atm H_2/acetone–EtOH/rt, 10 h, 100%; (e) estrone (2.01 equiv.)/THF/KOH/reflux 15 h, 65%; and (f) succinic anhydride (5 equiv.)/pyridine/reflux 12 h, 78%.



Scheme 3. (a) **3** and **4** (10 equiv.) DMAP/CH_2Cl_2/rt 24 h, 72%.

compound **2**, *trans*-1,3-*O*-benzylidene glycerol was first used to react with **5** to synthesize **6**. In acidic conditions, the acetal of **6** was decomposed leading to the exposure of two hydroxyl groups to form the important compound **7**. Because *trans*-1,3-*O*-benzylidene glycerol is more expensive than the *cis*,*trans*-1,3-*O*-benzylidene glycerol mixture, we later successfully used the mixture to react with **5** to efficiently get **7** in total 70% yield as described in supporting information. Compound **7** was tosylated to produce the oily diester **8** in 99.5% yield. The diester **8** was then hydrogenized to afford the oily diester **9** at almost 100% yield. The diester **9** reacted with estrone to give the di-estrogen compound **10** as a semi-solid. Although there is a hydroxyl group in compound **9**, with our successful selective reaction conditions, namely refluxing in THF with a KOH catalyst, only the phenolic hydroxyl group of estrone was active enough to react with the tosyl group of **9** to afford **10** (mp 108–110 °C). Thus, the side chain **4** was finally obtained through the reaction of **10** with succinic anhydride in pyridine to form a waxy solid in 78% yield.

The target compound DEC **11** was successfully synthesized through the convergent reaction of **3** and **4**, as shown in Scheme 3. The most difficult challenges in this final assembly were obtaining a suitable solvent and making all the 8-hydroxyl groups in **3** react completely to avoid any ''residue'' hydroxyl groups. The difficulties came from the solubility of **3** and **4**, because **3** was more soluble in polar solvents and **4** was soluble in non-polar solvents. According to our research, CH_2Cl_2 provided the perfect solvent for this reaction, because **3** was very slightly soluble in CH_2Cl_2 and gradually disappeared from the reaction mixture as the reaction proceeded. Compound **11** was obtained as a pure white solid (mp 65–67 °C) at 72% yield. The overall yields of the 9 steps required to synthesize DEC **11** is 16.5%. Compounds **2**, **3**, **4**, **6**, **7**, **8**, **9**, **10** and **11** have not been reported previously. Their preparation processes and structural characterizations were described in supporting information.

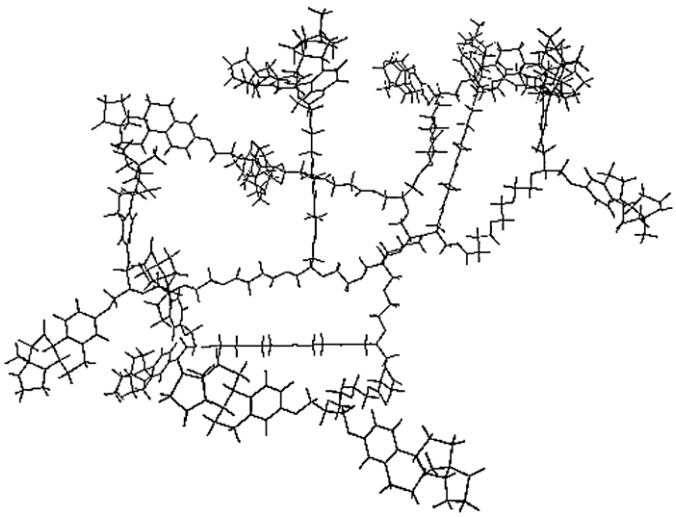


Fig. 2. Three-dimensional model of the dendrimer **11**.

The three-dimensional molecular model of the novel estrogen dendrimer **11** was simulated using ChemBio3D software (Fig. 2). This picture shows the flexibility of DEC and the full exposure of the estrogen, suggesting its advantages and potential use for studies of nuclear and extranuclear pathways of estrogen activity. Further studies of its biological activities will be reported in due course.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2012.11.009.

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