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Synthesis of novel *N*-diazeniumdiolates based on hyperbranched polyethers

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Abstract—Novel *N*-diazeniumdiolate based on hyperbranched polyethers(HP-g-DACA/N_2O_2) were prepared through a two-step synthesized route. The alkyltrimethoxysilane containing secondary amine groups (DACA) was used to modify the hydroxyl end groups of hyperbranched polyethers (HP) to obtain the precursor hyperbranched diamine (HP-g-DACA). Then HP-g-DACA was reacted with NO at 80psi pressure to be converted into *N*-diazeniumdiolates. The structures were confirmed using ^{13}C NMR and IR spectra. UV–vis spectroscopy measurement indicated that the aqueous solution of obtained HP-g-DACA/N_2O_2 had a characteristic absorption at 246 nm. The final HP-g-DACA/N_2O_2 product showed NO releasing within the prolonged periods of time, and the apparent half-life $t_{1/2}$ was more than 11 min in phosphate buffer at 37 °C. The total amount of NO released from HP-g-DACA/N_2O_2 could achieve to 0.43 μmol/mg and was proportional to the modified degree of HP by DACA. In addition, the NO loading efficiency can be modulated by the modification degree of hyperbranched macromolecular end groups.

Nitric oxide (NO) is an important bioregulatory agent and is endogenously produced by human endothelial cells to prevent platelet adhesion and activation, decrease the propensity of thrombus formation,[1–4] reduce bacterial adhesion[5], and kill cancer cells.[6] Recently, the synthesis of compounds that will store NO is of great interest due to its potential to serve as a source of nitric oxide (NO) under a wide variety of conditions. Compared with other organic NO donor species reported to date, *N*-diazeniumdiolate (NONOates) are more attractive candidates studied by many research groups since they undergo slow decomposition and decompose spontaneously under physiological conditions to regenerate NO.[7,8] N-Diazeniumdiolate NO donors are prepared by the reaction of amines with NO at certain pressure.[9,10] The first reported *N*-diazeniumdiolate was the NO adducts of diethylamine (DEA/NO) prepared by Drago and co-workers in 1960,[11] but it was really ignored until the past decade when the central role of NO chemistry in biology has emerged. The release of NO from *N*-diazeniumdiolate is widely depending on the chemical structure of the amine precursor and the pH of the reaction medium.[8,12] The dissociation reaction follows the simple pseudo-first-order rate laws, but in some cases it has been found to be markedly effected by the formation of bimolecular complexes at higher concentrations and by the presence of metal ions. The half-life of diazeniumdiolates varies from 1.8 s for PROLI/NO[13] to 20 h for DETA/NO[9] as determined at 37 °C in pH 7.4 of phosphate buffer.

More recently, the development of diazeniumdiolate NO donors with enhanced nitric oxide loading on a single molecule to produce materials capable of storing large quantities of NO has been achieved. For example, Reynolds et al. demonstrated that the reaction of symmetrical dialkyldiamine compounds with NO can generate doubly loaded diazeniumdiolate adducts. The resulting compounds can release 4 mol of NO per mole of original diamine.[14] However, the NO loading and duration properties of macromolecular *N*-diazeniumdiolate are not received the improvement. As these *N*-diazeniumdiolate applied in the polymer matrix, the secondary amine precursor of *N*-diazeniumdiolate is prone to leak into the aqueous solution to form carcinogenic nitrosamine.[15] As for macromolecular *N*-diazeniumdiolate, the leakage is much smaller than the relevant small molecular *N*-diazeniumdiolate due to its stronger interaction with the polymer matrix.[16] Therefore, it is meaningful to

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Keywords: Nitric oxide; *N*-Diazeniumdiolates; Hyperbranched polyethers; Modification.

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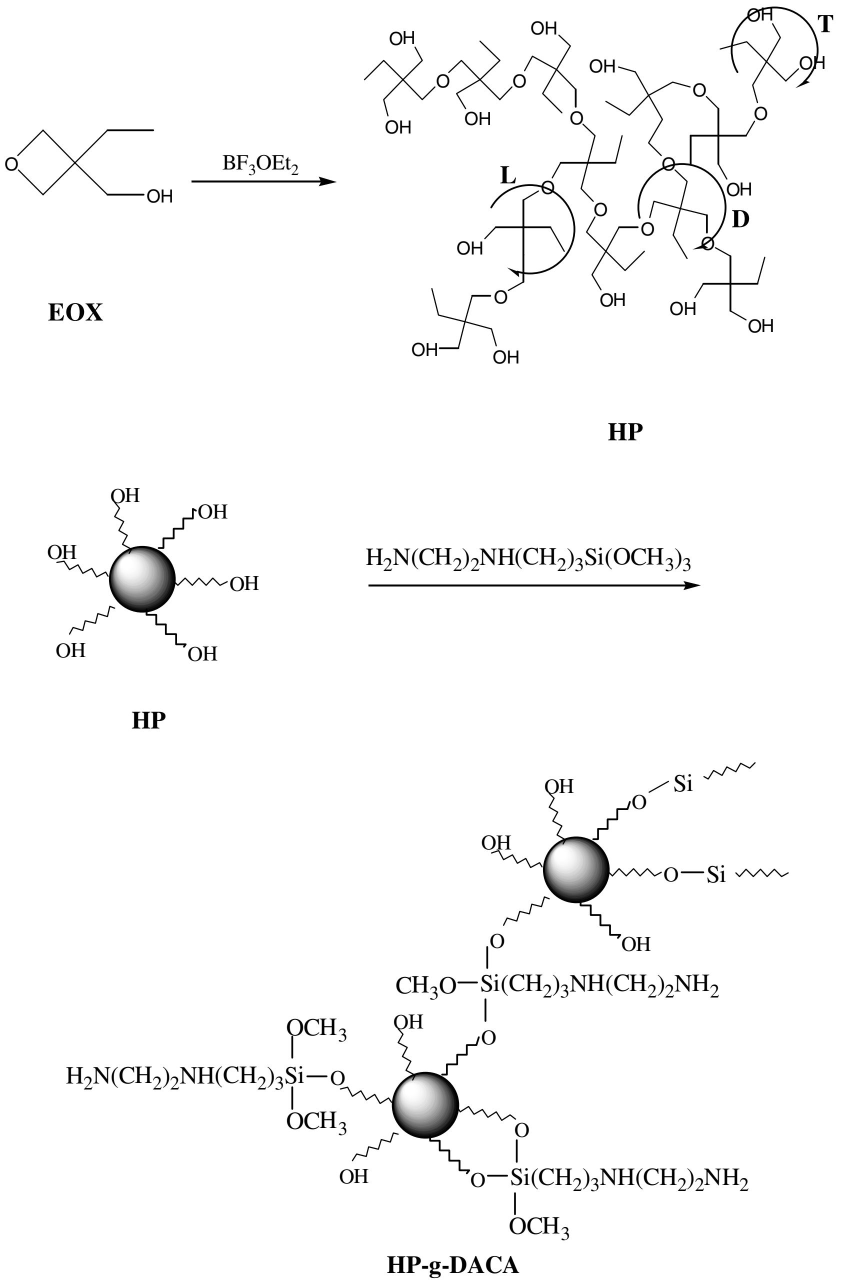


Scheme 1. Schematic synthesis of HP-g-DACA.

prepare a macromolecular *N*-diazeniumdiolate with higher NO loading and slower NO releasing properties.

At present, dendritic polymers containing dendrimer and hyperbranched polymer, have received significant consideration due to their unique molecular structure and excellent properties as compared with their linear analogues.[17,18] Most important, a large number of end groups of dendritic polymers offer the possibility for further modification of terminated groups to fine tune their properties for special applications.[19] However, the dendrimer has well-defined molecular structure and be prepared by multistep reactions with laborious isolation and purification process, which is very expensive and limited its large scale synthesis and application. And the molecular structure of hyperbranched polymer is not perfect and can be synthesized in simple 'one-pot' procedure that suit for large scale synthesis and application. In many cases, hyperbranched polymer is discussed as a more rapidly prepared and more economical replacement of perfect dendrimer.

This communication reported the first example for the preparation of *N*-diazeniumdiolate based on hyperbranched polyethers (HP) using *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane (DACA) as the end group modifier to provide the required secondary amine sites necessary to generate *N*-diazeniumdiolate moieties. The formation of *N*-diazeniumdiolate was confirmed

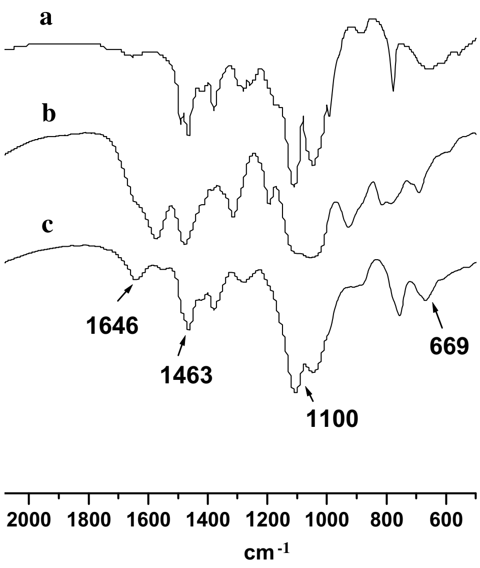


Figure 1. IR spectra of HP (a), DACA (b), and HP-g-DACA (molar ratio HP/DACA = 1:5) (c).

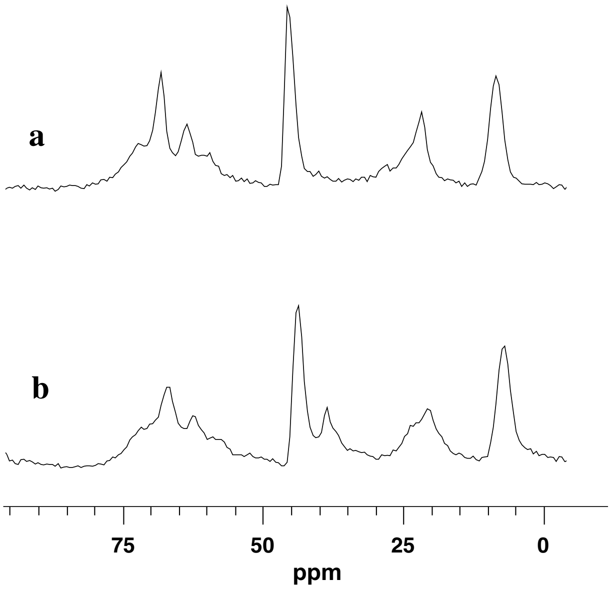
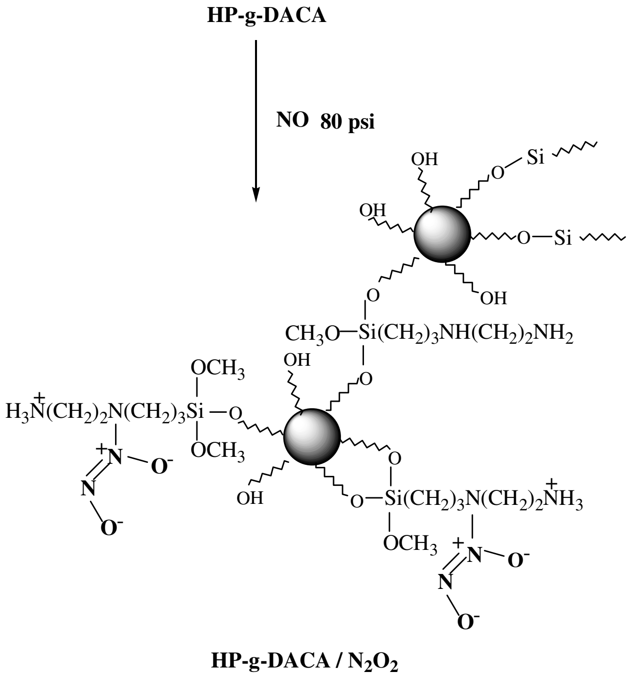


Figure 2. Solid state ^{13}C NMR of HP (a) and HP-g-DACA (molar ratio HP/DACA = 1:5) (b).

to be successful. In addition, the NO loading efficiency of modified hyperbranched polyethers (HP-g-DACA) can be modulated by the modification degree of end groups.

The modified hyperbranched polymer (HP) was prepared by cationic ring-opening polymerization of monomer 3-ethyl-3-oxetanemethanol (EOX) using BF_3OEt_2 as a catalyzer and DACA as a modifier (Scheme 1). The modified polymer (HP-g-DACA) was characterized by solid-state ^{13}C NMR and FT-IR to determine the covalent linkage of secondary amine of the molecular backbone of hyperbranched polyether. Figure 1 shows the IR spectra of HP (a), DACA (b), and HP-g-DACA (c). As shown in Figure 1c, the absorption peak at 1646 cm^{-1} is attributed to the stretching of $-NH_2$, just approximately similar to the absorption that exists in Figure 1b. The results of solid state ^{13}C NMR (in Fig. 2) have demonstrated that, in comparision of the ^{13}C NMR spectrum of HP-g-DACA with that of HP, the additional resonance signal at 39.5 ppm is contributed to $-CH_2-NH_2$ group, indicating the covalent attachment of DACA to the molecular structure of HP. Both characterizations of FTIR and ^{13}C NMR have confirmed the successful modification of HP to obtain amine-functionalized hyperbranched polyether for the further reaction with NO to prepare *N*-diazeniumdiolate.

The *N*-diazeniumdiolate based on hyperbranched polyether was synthesized through the exposure of DACA modified HP (HP-g-DACA) to NO flux at 80 psi pressure (Scheme 2). UV–vis spectroscopy was used to confirm the formation of diazeniumdiolate-NO donor within the hyperbranched matrix. Figure 3a illustrates the UV–vis spectrum of the freshly prepared diazeniumdiolate as dispersed in the PBS solution. It was reported that the *N*-diazeniumdiolates of primary amine has an absorption maximum at λ_{max} 252 nm, while the corresponding second amine *N*-diazeniumdiolates has an absorption maximum at λ_{max} 246 nm[15]. For the amine used in the present study, both primary and secondary amino nitrogens are contained in the molecular structure. As can be seen in Figure 3a, the λ_{max} is found to be 246 nm and indicates that the *N*-diazeniumdiolate groups were predominantly linked to the secondary amino nitrogen in the proceeding of NO-addition. The result also demonstrates that the secondary amine is more favorable for reaction with NO



Scheme 2. Synthesis scheme for preparing *N*-diazeniumdiolate based on hyperbranched polyether HP-g-DACA/N_2O_2.

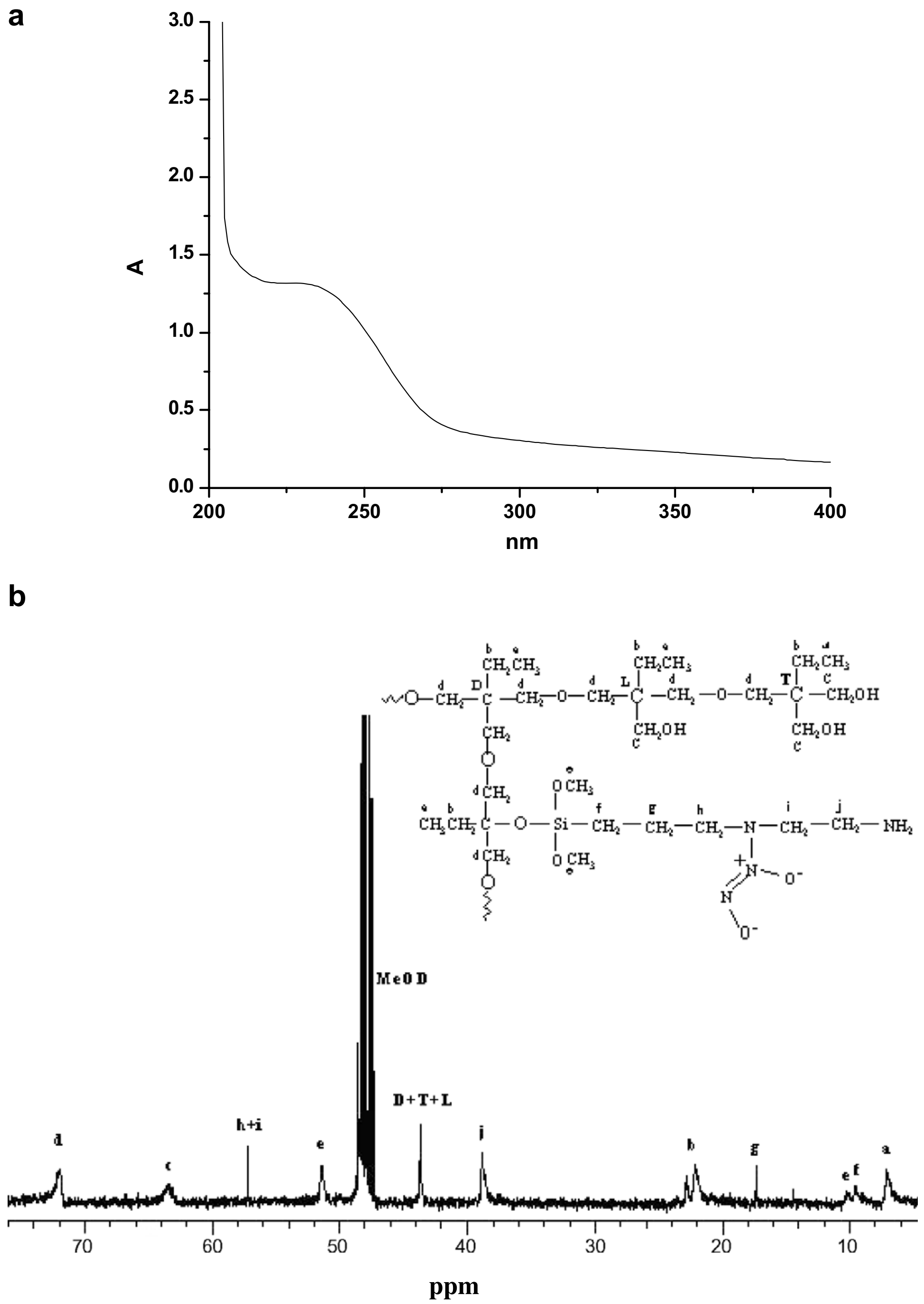


Figure 3. Example of decomposition of HP-g-DACA/N_2O_2 as determined by UV spectroscopy at room temperature (a) and ^{13}C NMR spectrum of HP-g-DACA/N_2O_2 (b). Molar ratio HP/DACA = 1:5.

than the primary amine sites. This observation is consistent with previous results reporting the exclusive NO-addition with the secondary amino nitrogen of some triamines (e.g., diethylenetriamine).[15] In addition, the successful preparation of novel diazeniumdiolate HP-g-DACA/N_2O_2 could be monitored by ^{13}C NMR in MeOD as shown in Figure 3b. It demonstrated that the signal at 57.2 ppm was contributed to the carbons (**h+i**) near the nitrogen to which the N_2O_2 group was attached. This result was in good agreement with that of Red'kin et al.[20] Furthermore, the characterized chemical shifts of the carbons (**g** and **j**) appeared at 18.8 ppm and 39.3 ppm, which were shifted upfield as the relative carbons in DACA appeared at 23.3 ppm and 41.9 ppm[21]. On the other hand, the indirect measurement of NO release using griess reagent spectrophotometric method further confirmed that diazeniumdiolates were indeed formed within the hyperbranched macromolecules upon exposure to NO.

In the present experiment, the total amount of NO released from HP-g-DACA/N_2O_2 diazeniumdiolates

Table 1. The preparation and properties of HP-g-DACA/N_2O_2

Sample	HP/DACA (molar ratio)	$t_{1/2}$ (min)	$t_{[NO]}$[a] (μmol/mg)	NO-addition efficiency[b] (%)
HP-g-DACA/N_2O_2-5	1:5	9	0.24	23
HP-g-DACA/N_2O_2-10	1:10	6.7	0.35	27
HP-g-DACA/N_2O_2-20	1:20	11	0.42	33
HP-g-DACA/N_2O_2-30	1:30	6.5	0.43	25

[a] Measured indirectly by Griss assay in phosphate buffer (pH 7.4) at 37 °C.

[b] Calculated according to the following equation: NO-addition efficiency $= \frac{\text{NO release(μmol)}}{\text{μmol of DACA}\times\text{2mol of NO}} \times 100\%$.

varied with the concentration of DACA relative to total hyperbranched molecular structures. It was found that NO loading increased with the content of loaded DACA when molar ratio of DACA/HP was lower than 20, as illustrated in Table 1. However, the amount of NO released changed very little as the molar ratio of DACA/HP exceeded 20, as could be seen by comparing the total amount of NO released values for the DACA/N_2O_2-20 and DACA/N_2O_2-30 samples. The produced HP-g-DACA/N_2O_2 showed NO releasing within the prolonged periods of time, and the apparent half-life $t_{1/2}$ was more than 11 min in phosphate buffer at 37 °C. The total amount of NO released from HP-g-DACA/N_2O_2 could achieve to 0.43 μmol/mg and was proportional to the modified degree of HP by DACA. The maximum total NO loading of HP-g-DACA was much larger as compared with that of corresponding linear polymer.[22] As the number of amine groups in the hyperbranched matrix was increased with the higher modification of end groups by DACA, the number of sites available for diazeniumdiolate formation was also increased, which was an advantage for the enhancement of NO loading efficiency. However, with the increase of DACA content to a certain degree, the increase of diazeniumdiolate formation was not obvious. Due to the unique spherical molecular structure of hyperbranched polymer, as the amount of DACA in the molecule reached a degree, the spatial hindrance for the reaction of NO with the amine was increased. Therefore, the addition of NO was interrupted to obtain *N*-diazeniumdiolates. The results demonstrated that the NO loading efficiency of this novel hyperbranched polyether *N*-diazeniumdiolate can be regulated by the modification degree of end groups by DACA.

In summary, the synthesis of a novel *N*-diazeniumdiolate based on hyperbranched polyether was investigated. The results clearly demonstrated the formation of NONOates groups on the hyperbranched macromolecular structure. The obtained *N*-diazeniumdiolate can release NO up to 0.43 μmol/mg, which was much larger than that of linear polymer. It indicated that the NO loaded efficiency of HP-g-DACA can be enhanced due to the unique molecular structure of hyperbranched polymer. Furthermore, the variation of modification degree of end groups gave the modulation of NO loading efficiency of hyperbranched polymers. The decomposition properties of *N*-diazeniumdiolate, which were essential aspects for the potential application as NO donors, will be discussed in a forthcoming full paper.

Acknowledgments

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