



Highly efficient synthesis and antioxidant activity of O-(aminoethyl)inulin

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ABSTRACT

O-(aminoethyl)inulin was prepared from inulin using ethylene imine, a kind of significant building block of chemical modification. The preparation of the O-(aminoethyl)inulin was studied in such catalytic systems as: water (H₂O)/sodium hydroxide (NaOH), N-methylpyrrolidinone (NMP)/triethylamine (Et₃N) and benzene/anhydrous alcholor (AlCl₃). Each of these systems was evaluated in terms of reaction yield rate and degree of substitute. Of all the reaction systems, the H₂O/NaOH method turned out to be the most recommended means for the preparation of the O-(aminoethyl)inulin with a yield rate 82% and 0.76 degree of substitute. Additionally, the antioxidant effects of inulin and its related derivatives of different degree of substitute were investigated in two model systems. Comparing with inulin, modified inulin exhibited a moderate hydroxyl radical scavenging ability and considerable superoxide-radical scavenging activity. And this may suggest their potential employment as a prohibitor of the superoxide-radical. The synthesized derivatives were characterized by ¹HNMR and FT-IR spectra.

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1. Introduction

Inulin, the reserve polysaccharide of several plants, consists primarily of β(2–1) fructosyl fructose units (F_m) – always presented in furanose form – with commonly a reducing end (GF_n) formed in an individual glucopyranose unit (Fig. 1) (Stevens, Meriggi, & Booten, 2001). This structural singularity raises its interesting properties like beneficial nutritional attributes, which in turn excites the chemical modification of inulin in recent years (Beylot, 2006). We could get inulin that have an excellent average degree of polymerization (DP) according to the type of plants from which inulin was extracted (Stevens et al., 2001; Wei et al., 2007). The unique interesting properties and ready access of inulin, together with its excellent DP, suggest the wonderful potential that the inulin could be widely employed in various aspects of function food and other ways.

In recent years, great deals of efforts are being devoted to the industrial utilization of this extraordinarily abundant renewable natural product via means of chemical modification (Dan, Ghosh, & Moulik, 2009; Rogge, Stevens, Colpaert, Leveck, & Booten, 2007). In a review, Stevens provided us with a relatively integral overview of various ways of modification of inulin and their probable industrialized application fields (Stevens et al., 2001). One can easily find

that the majority of the chemical modifications of inulin stated in the review and other concerned literatures are mostly by ways of ethers or esters involving the hydroxyl group of the inulin (Stevens et al., 2001). Though there are lots of industrial employments of this renewable natural product via chemical modification stated in studies recently, they are comparatively insufficient when compared to the utilization of other polysaccharides, for instance the chitosan, and when the wonderful biological activities of the inulin revealed in numerous investigations are taken into account (Beylot, 2006; Causey, Feirtag, Gallaher, Tungland, & Slavin, 2000; Taper & Roberfroid, 1999). Thoroughly studying the industrialized utilization of the chitosan, we can note that nearly all of the chemical modification of the chitosan cannot be separated from the relatively active amino group on the chitosan which is being used to form Schiff base (–C=N), secondary amine (–NHR) and so on (Guo, Liu, Chen, Ji, & Li, 2006), which gives us the inspiration that active amino groups on the inulin are desirable. For this end, we set out to find a readily efficient way to synthesize a kind of inulin derivatives with active amino groups on them.

As mentioned in previous paragraph, most of the chemical modifications of inulin are through ester or ether bonds and the latter bonds are far stronger than the former when they encounter acid or base in a chemical reaction. There are literatures reporting that inulin could react easily with ethylene oxide (Rogge et al., 2005). Given these characteristics of reactions of inulin, combined with the demand that the synthesis should be facilitated and efficient since we are just to synthesize a precursor expected to go through a series of reaction, we selected the ethylene imine, a significant building block for chemical bond elaborations and could be

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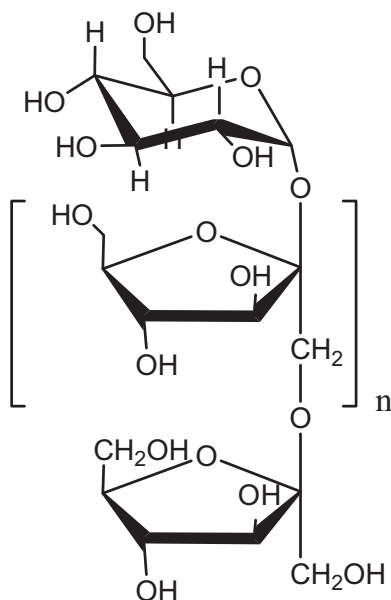


Fig. 1. Structure of average native inulin (GF_n).

conveniently prepared in laboratory following Wenker's method (Wenker, 1935), as our start material to synthesize the desired derivatives.

Polysaccharides extracted from plants have exhibited excellent antioxidant properties, which can be developed as novel potential antioxidants (Hu, Xu, & Hu, 2003; Tseng, Yang, & Mau, 2008). However, up to now, the antioxidant activity of inulin has not been reported. In this paper, we evaluated and compared the antioxidant activity of inulin and the synthesized derivatives.

2. Materials and methods

2.1. Materials

Inulin was purchased from Wede biological Corp. (Beijing, China) and was employed without further purification. Its average degree of polymerization was around 20 fructosyl fructose units. Sodium hydroxide (NaOH), triethylamine (Et_3N), benzene, and anhydrous aluminum chloride (AlCl_3) were purchased from the Sigma–Aldrich Chemical Co. Other reagents were all analytical grades and, unless otherwise specified, were used as received. The FT-IR spectra were measured on a JASCO-4100FT-IR spectrometer. The samples were prepared as KBr pellets and were scanned against the air background at room temperature and the ^1H NMR spectra were measured with a Bruker AVIII-500 spectrometer operating at 500 MHz, 25°C , in $\text{DMSO}-d_6$.

2.2. Syntheses

The inulin derivative O-(aminoethyl)inulin (OAEIL) was according to Fig. 2. Tables 1 and 2 show the synthesis conditions of the compound. And X was the molar ratio that the ethylene imine compared with inulin.

2.2.1. General synthesis of OAEIL in water with NaOH as catalyst

1.62 g of inulin (10.0 mmol of fructose equivalents) was added to 12 mL 40% NaOH solution at 25°C . After stirred at room temperature for 4 h, the mixture was frosted at -20°C for 8 h, and then the temperature was raised to 75°C , X molar equivalent ethylene imine (prepared by Wenker's method) of fructose diluted to 5% by distilled water was added dropwise to the mixture. Under stirring the

solution was reacted for 16 h at 75°C . The mixture was precipitated in 200 mL methanol and was filtered by suction. The inulin derivative was washed with acetone, dichloromethane and was dried at -50°C in vacuum for 24 h.

2.2.2. General synthesis of OAEIL in NMP with Et_3N as catalyst

1.62 g of inulin (10 mmol of fructose equivalents) was dissolved in 10 mL of NMP at 60°C and 0.72 mL Et_3N (0.5 equiv. based on fructose equivalents) was added. When temperature was raised to 85°C , the reaction was continued for 3 h at this condition with stirring. Then, the reaction solution was cooled to 75°C and X molar equivalent ethylene imine (prepared by Wenker's method) of fructose diluted to 5% by NMP was added dropwise to the mixture. After the reaction was heated at 75°C for 24 h, it was poured into 200 mL acetone and the product crystallized easily. The synthesized OAEIL was, washed with acetone and dichloromethane, dried at -50°C in vacuum for 24 h.

2.2.3. General synthesis of OAEIL in benzene with AlCl_3 as catalyst

To a solvent of 15 mL dry benzene (distilled over CaH_2), 0.14 g AlCl_3 was slowly added at 0°C and the mixture was further stirred at this temperature until homogeneous. Then 1.62 g of inulin (10 mmol of fructose equivalents and dried at 60°C overnight in vacuum) was added and when the temperature was raised to 85°C , 12.42 mL 5% ethylene imine was added dropwise to the mixture. Under stirring, the solution was refluxed for 16 h at 85°C . The mixture was filtered by suction and the derivatives were washed and dried.

2.3. The investigation of the antioxidant ability

2.3.1. Hydroxyl-radical scavenging ability assay

The test of the hydroxyl-radical scavenging ability was carried out according to Liu's methods (Liu et al., 2009). The reaction mixture, a total volume 4.5 mL, containing the samples of the inulin derivative, was incubated with $\text{EDTA}-\text{Fe}^{2+}$ ($220\text{ }\mu\text{M}$), safranine O ($0.23\text{ }\mu\text{M}$), H_2O_2 ($60\text{ }\mu\text{M}$) in potassium phosphate buffer (150 mM , pH 7.4) for 30 min at 37°C . The absorbance of the mixture was measured at 520 nm. Hydroxyl radicals bleached the safranine O, so increased absorbance of the reaction mixture indicated decreased hydroxyl radicals scavenging ability and the scavenging effect of the product was computed using the following equation:

$$\text{Scavenging effect (\%)} = \frac{A_{\text{sample } 520\text{ nm}} - A_{\text{blank } 520\text{ nm}}}{A_{\text{control } 520\text{ nm}} - A_{\text{blank } 520\text{ nm}}} \times 100$$

where $A_{\text{blank } 520\text{ nm}}$ was the absorbance of the blank (distilled water instead of the samples), $A_{\text{control } 520\text{ nm}}$ was the absorbance of the control (distilled water instead of the H_2O_2).

2.3.2. Superoxide-radical scavenging ability assay

The superoxide radical scavenging ability was assessed following the model of Xing (Xing et al., 2006). Involving testing samples ($0.05\text{--}0.8\text{ mg/mL}$), phenazine mothosulfate ($30\text{ }\mu\text{M}$), nicotinamide adenine dinucleotide reduced ($338\text{ }\mu\text{M}$), and nitro blue tetrazolium ($72\text{ }\mu\text{M}$) in phosphate buffer (0.1 M , pH 7.4), the reaction mixture was incubated at 25°C for 5 min and the absorbance was read at 560 nm against a blank. The capability of scavenging superoxide radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = 1 - \frac{A_{\text{sample } 560\text{ nm}}}{A_{\text{control } 560\text{ nm}}} \times 100$$

where $A_{\text{control } 560\text{ nm}}$ was the absorbance of the control (distilled water instead of the samples).

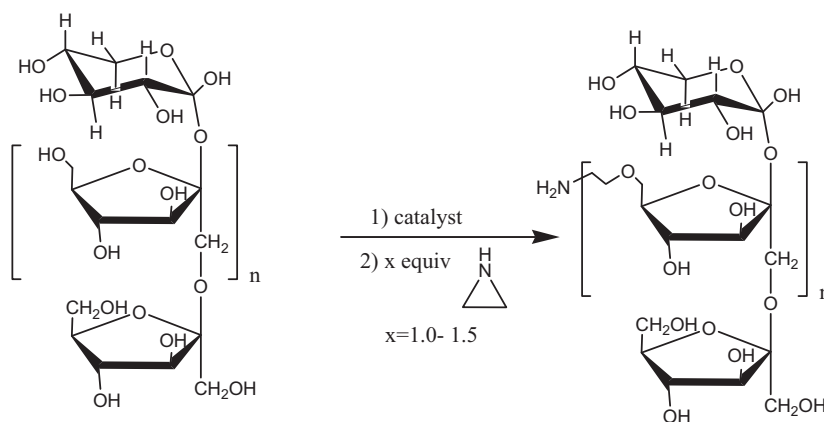


Fig. 2. Typical synthesis pathway of OAEIL.

Table 1

The synthesis data of the inulin derivative via NaOH/water method.

X ^a	1.0	1.1	1.2	1.3	1.4	1.5
Yield ^b	83%	78%	82%	80%	74%	82%
DS ^c	0.54	0.68	0.76	0.73	0.70	0.68

^a The molar ratio that the ethylene imine compared with inulin.

^b The reported yields are based on the amount of product recovered after crystallization in a non-solvent.

^c The reported DS was calculated by ¹HNMR.

2.4. Statistical analysis

All data are expressed as means \pm SD. Data were analyzed by an analysis of variance ($P < 0.05$) and the means were separated by Duncan's multiple range test. The results were processed by the computer programs: Excel and Statistica software SPSS.

3. Results and discussion

3.1. Chemical syntheses

The FT-IR spectra of inulin and a representative OAEIL (0.76DS) were shown in Fig. 3 and the ¹HNMR spectra of them were exhibited in Fig. 4. As shown in Fig. 3, the IR spectra present the comparison of transmission spectra data of OAEIL with that of original inulin. As for the FT-IR spectra was concerned: first, the big translocation between 3536 and 3236 cm⁻¹ with a peak at 3386 cm⁻¹ of original inulin changed to between 3520 and 3380 cm⁻¹ with a peak at 3400 cm⁻¹ of the inulin derivatives and at the same time, the breadth of the translocation of the product narrowed compared with the spectra of inulin. A reasonable explanation of these two shifts could be the single O–H group stretching vibration in inulin transformed to O–H group combining with N–H stretching vibration group in OAEIL and the decrease of hydroxy groups of the product, which were used to connect ethylene imine during reaction stage (Zhong et al., 2007). Second, the new peaks at 1592 cm⁻¹ and 1083 cm⁻¹ appeared at the spectra of the product, respectively suggesting the new group –NH₂ and the new ether bond

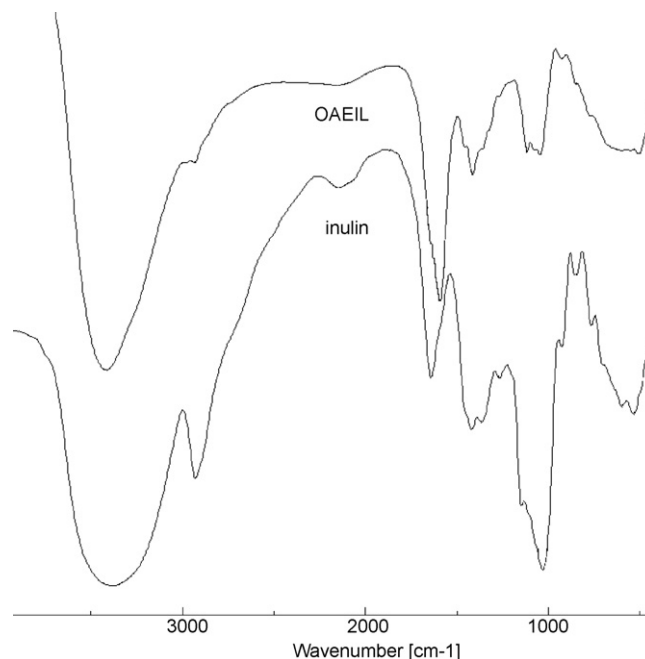


Fig. 3. FT-IR spectra of inulin and OAEIL.

Table 2

The synthesis data of the inulin derivative via Et₃N/NMP method.

X ^a	1.0	1.1	1.2	1.3	1.4	1.5
Yield ^b	90%	92%	90%	90%	93%	86%
DS ^c	0.17	0.19	0.16	0.20	0.17	0.14

^a The molar ratio that the ethylene imine compared with inulin.

^b The reported yields are based on the amount of product recovered after crystallization in a non-solvent.

^c The reported DS was calculated by ¹HNMR.

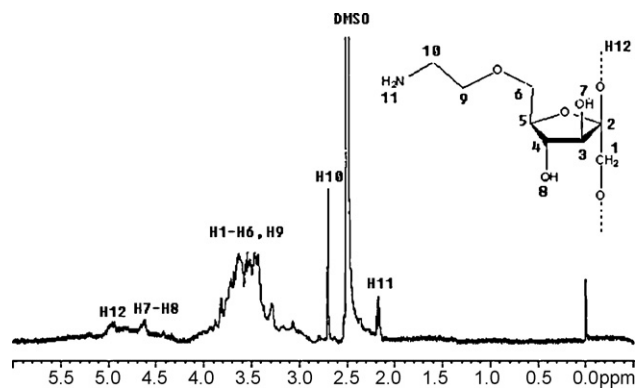
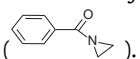


Fig. 4. ^1H NMR spectra data of OAEIL.

($\text{CH}_2\text{OCH}_2\text{NH}_2$) of the derivatives (Guo et al., 2006; Zhong et al., 2007). The structure of the OAEIL was further confirmed by ^1H NMR (Fig. 4). The chemical shifts at δ 2.2 (H-11) and δ 2.7 (H-10) were respectively assigned to NH_2 group and the $-\text{CH}_2$ adjacent to NH_2 group (Guo, Xing, Liu, Zhong, & Li, 2008). The above-mentioned results evidently substantiated the obtaining of the aimed derivatives.

Although certain literatures and patents have revealed that the inulin could react easily with ethylene oxide in the absence of catalyst (Rogge et al., 2005), yet according to our study, no such reaction occurred between inulin and ethylene imine due to the weaker electronegativity of nitrogen than that of oxygen. We have also attempted to activate the reaction with the help of benzoyl chloride. Firstly, let benzoyl chloride react with ethylene imine to form

aziridin-1-yl-(phenyl)methanone () . Then inulin could directly perform nucleophilic ring opening of aziridin without the existence of catalyst due to the electron withdrawing effect of benzoyl group. However, hydrolysis of the amide to release amino group was proved to be a rather tough job and the hydrolysis was inevitably conducted at the expense of the reaction efficiency. Fortunately, as we expected, the inulin could readily perform the nucleophilic ring opening of ethylene imine with the help of some base.

To develop an elegant and high efficient method that featured by good DS and yield rate, we studied the reactions in depth (Fig. 2). The DS of modified inulin could be calculated according to ^1H NMR by comparing the integration of signal of the $-\text{NH}_2$ groups (at about 2.1 ppm) to a specific signal of fructose unit integrating for the 3-OH and 4-OH groups at 4.5–4.6 ppm (Fig. 4). The yield rate is based on the amount of desiccated product recovered after crystallization and the integrated amount of start material. The results of the yield rate and DS of the derivatives via NaOH/water and $\text{Et}_3\text{N}/\text{NMP}$ methods were listed in Tables 1 and 2. From Table 1 we could easily see that when performed in aqueous medium, with NaOH as catalyst, the reaction gave products that had relatively good DS ranging from 0.54 to 0.76, and which may benefit from the frosted phase. During the solution was frosted at -20°C , the water among inulin was iced over and this could help to break the hydrogen bond inside the inulin molecule largely and expose 6-OH groups of inulin. However, due to comparatively weak base attribute, the Et_3N cannot do the job as well as NaOH (Table 2). Though it gave a good yield rate (up to 93%), the DS was too low which was fluctuating around 0.17. Therefore, the catalyst Et_3N seems to be not suitable for the synthesis of OAEIL, a modification precursor. When the reaction was catalyzed by Lewis acid AlCl_3 , appropriate solution was out of sight and the reaction had to be conducted in heterogeneous phase, which inevitably led to a rather low efficiency.

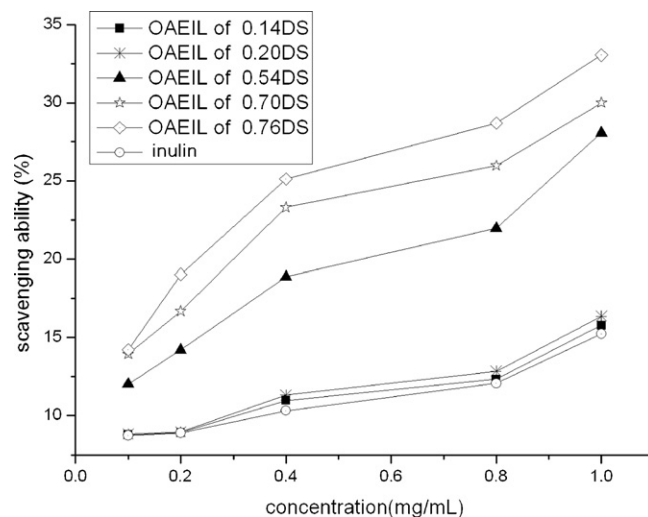


Fig. 5. Hydroxyl radical scavenging ability of OAEIL and inulin.

Unfortunately, the DS of the reaction was too low to be computed by ^1H NMR.

3.2. Antioxidant activities

3.2.1. Hydroxyl radical scavenging activity of OAEIL and inulin

Under normal condition, direct addition of Fe^{2+} to a reaction mixture containing phosphate buffer generates hydroxyl radicals (Halliwell & Gutteridge, 1990) which are harmful to body through reacting with such biological molecule as amino acids or DNA. Fig. 5 reveals the $\cdot\text{OH}$ scavenging ability of inulin and OAEIL with different DS of 0.14, 0.20, 0.54, 0.70 and 0.76. We can see that all the scavenging effects of samples have positive correlation with the DS and concentration. Although the derivatives just exhibited a relatively moderate scavenging ability, the best of which was approaching 35% at 1 mg/mL (the OAEIL of 0.76DS), they are far better than the original inulin with a scavenging effect of no more than 14% at 1 mg/mL. Considering that OAEIL is only developed as an easy going chemical modification precursor via a simple and highly effective reaction, the scavenging index is considerable. It is reasonable to propose that the enhanced scavenging capability may benefit from the NH_2 grafted on inulin. The NH_2 groups can form ammonium groups NH_3^+ by absorbing hydron from solution, and then react with $\cdot\text{OH}$ (Xie, Xu, & Liu, 2001). In addition, the higher scavenging index may have something to do with N atom since it have weaker electronegativity than O atom, which makes the NH_2 of the derivatives more reactive when it encounters radicals like $\cdot\text{OH}$ than the original OH of inulin used to link ethylene imine.

3.2.2. Superoxide-radical scavenging activity of OAEIL and inulin

Superoxide is another kind of deleterious molecule to the body. Even though it exhibits limited chemical reactivity, it can generate more dangerous species such as: singlet oxygen or hydroxyl radicals, which will cause the peroxidation of lipids (Xing et al., 2006). Fig. 6 gave the curve chart of the superoxide-radical scavenging effect of the modified inulin with various DS of 0.14, 0.20, 0.54, 0.70 and 0.76 on series concentration levels ranging from 0.1 to 1 mg/mL. According to the graph we could conclude the results as follows: firstly, unlike on the previous hydroxyl-radical scavenging effect, this time, all the tested objects, especially the derivatives, showed a marked inhibitory effect on superoxide-radical. Secondly, the scavenging rate increases with increasing concentration and DS. Again, of all the compounds, OAEIL of 0.76 DS exhibited the best activity. IC_{50} value of OAEIL of 0.76 DS and inulin was 0.52

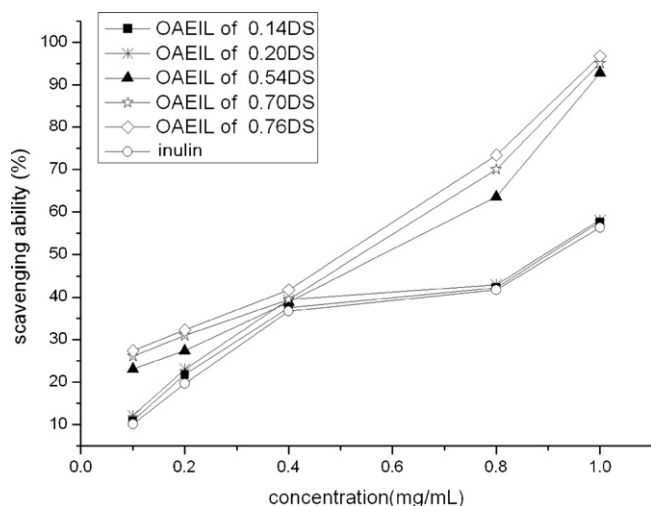


Fig. 6. Superoxide-radical scavenging ability of OAEIL and inulin.

and 0.94 mg/mL, respectively. IC_{50} , a good parameter to evaluate the scavenging activity, means the modified inulin concentration to reduce the radical by 50%. Thirdly, significant scavenging effect (72.08–83.74%) of superoxide radicals was evident at tested concentrations of OAEIL, which suggests the potential of the product to be developed as a kind of superoxide-radical scavenging reagent of food industry. Once more, the grafted $-NH_2$ may be helpful to the scavenging ability on superoxide-radical as the $-NH_2$ is more reactive than $-OH$ group. Also, the severely disrupted inner structure of inulin by the introduction of ethylene imine as well as the declined hydrogen bond among inulin during the frozen phase make the derivatives have better scavenging effect on superoxide-radical as these let the hydroxyl group bare and be ready to react with superoxide.

4. Conclusion

In summary, OAEIL derivatives were obtained by the first grade hydroxy groups of inulin performing nucleophilic ring opening of ethylene imine with the help of catalyst. In this manuscript, the described reaction of inulin has been studied in aqueous medium and organic solvents combined with different catalyst. For the evaluation of the different reaction system, to get good DS product, the reaction favors to be conducted in aqueous medium with NaOH as catalyst, since the base NaOH is far stronger than Et_3N and consequently gives a better basification degree which benefits the DS of the OAEIL. In addition, the antioxidant effect of inulin and the relative derivatives was investigated in two model systems. The data obtained in vitro models clearly suggest the antioxidant potency of the substances. The mechanism for the enhanced antioxidant

activities of OAEIL was also discussed in this paper. The antioxidant activities are probably influenced by the grafted NH_2 groups in the OAEIL derivatives, which were more active than the original OH groups when react with super-radicals.

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