Journal of the Korean Chemical Society
2011, *Vol. 55, No. 6*
Printed in the Republic of Korea
http://dx.doi.org/10.5012/jkcs.2011.55.6.978

Synthesis and Characterization of Lactobionic Acid Grafted Phenylalanyl-Glycyl-Chitosan

He-Ping Li*, Shan Li, Zhou-Dong Wang, and Long Qin

*College of Chemical and Biologic Engineering, Changsha University of Science and Technology, Changsha 410004, China. *E-mail: lihp@csust.edu.cn*

(Received June 29, 2011; Accepted September 14, 2011)

ABSTRACT. In order to enhance the target action of chitosan-based drug, this paper firstly prepared phenylalanyl-glycyl-chitosan (Phe-Gly-CS) by grafting the key intermediate, bromoacetyl-phenylalanine (BA-Phe) onto chitosan. Then the target sugar molecule, lactobionic acid (LA), was grafted to Phe-Gly-CS and the topic compound lactobionic acid grafted phenylalanyl-glycyl-chitosan (Phe-Gly-CS-LA) was finally obtained in a yield of 78.8%. The product were characterized by FT-IR, MS and 1H NMR. The preparing condition of BA-Phe was optimized as follows: the best pH was 10-11, the optimum temperature was -4 °C, the reaction time was 1.5 h.

Key words: Grafted chitosan, Drug carrier, prepare, Lactobionic acid

INTRODUCTION

Chemotherapy for cancer has been developed and improved over last half century, till now it is still one of the major treatment ways. However, most anticancer drugs have severe side-effects, which limited their clinical applications.[1] According to the principle of pharmacokinetics, the cure benefits have some connections with the drug concentration in the action site and time available for drug absorption.[2] In order to improve the efficiency of pharmaceutical agents, the targeted drug delivery systems that release drugs at the desired site of action, reduce therapeutic dose and toxicity, has attracted many chemical researchers' attention in recent years.[3]

Chitosan, a linear aminopolysaccharide composed of randomly distributed (1→4) linked D-glucosamine and N-acetyl-D-glucosamine units,[4] has prompted the impetus for the development of safe and effective drug delivery systems due to its good documented biocompatibility, low toxicity, and degradability by human enzymes.[5] It has been widely utilized as drug delivery systems for low molecular drugs, peptides and genes.[6]

At present, the majority of therapeutic drugs are primarily low molecular weight drugs which are being developed and marketed.[7] Thus, it is very important to successfully deliver the low molecular drugs to their respective targets in therapeutics. In this paper, we focused on synthesis of a new target-specific drug carrier based on chitosan. A series of chemical modifications of chitosan were conducted as follows: Firstly, through a reaction between bromoacetyl bromide and phenylalanine, BA-Phe was prepared to be grafted onto the C2-amino group of CS, which could improve the hydrophilicity and the stability of CS in an acid-base condition. Then the synthetic conditions of BA-Phe were optimized since it is a key intermediate compound in the whole synthesis. Then, lactobionic acid, a proposed ASGPR recognized sugar molecule, was further introduced into C2-amino group of dipeptide chitosan via an amide linkage. The structures of all compounds were characterized by IR, MS or ^{1}H NMR.

EXPERIMENTAL

Synthesis of BA-Phe

0.4 g (2.5 mmol) phenylalanine (Phe) and 0.1 g (2.5 mmol) NaOH were dissolved in distilled water, and the mixture was stirred for 0.5 h with an salt-ice-water bath. 10 mL toluene which contained 0.6 g (3.0 mmoL) bromoacetyl bromide was added dropwise. And the mixture was allowed to stir until the reaction was complete. In the whole process, addition of 2 mol·L^{-1} NaOH solution was to keep pH within the range of 10~11. After the reaction completed, the mixture was filtered, 36% HCl added to the filtrate for PH=1,[8] freezed at 4 °C for 12 h, filtered again, the white solid was washed 3 times with ice-water, then evaporated in vacuo. The yield was 78.8%, m.p: 111~112 °C. IR(KBr): 1719 cm^{-1} (C=O stretching of -*CO*O), 1621 cm^{-1} (C=O stretching of -*CO*N*H*), 1340 cm^{-1} (C-H axial deformation of Br-*CH*$_2$-), 637, 612 and 586 cm^{-1} (C-Br trans and cis stretching); MS: 308.1 (M+Na)$^+$.

Synthesis of Phe-Gly-CS

0.1130 g (0.7 mmol of -NH_2) chitosan was dissolved in 60 mL (0.025 mol·L^{-1}) HCl containing 0.4 g (1.4 mmol) BA-Phe, a prescribed amount of $NaHCO_3$ was then added to keep PH within the range of 8~8.5 and the reactions were allowed to proceed at 60 °C under magnetic stirring for 0.5 h. Then a catalyst amount of KI was added, continue stirring for 12 h.[9] The products were purified by dialysis against deionised water for 3 days, and then freeze-dried. White sponge solid was obtained. IR(KBr): 3347 cm^{-1} (*-OH* and *N-H*), 1641 cm^{-1} (*C=O* of *-CO*N*H*), 1538 cm^{-1} (*C=C* of benzene ring), 1384 cm^{-1} (*O-H*), 751 and 703 cm^{-1} (*C-H* of benzene ring).

Synthesis of Phe-Gly-CS-LA

0.2010 g (0.56 mmol) Lactobionic acid, 0.1075 g (0.56 mmol) EDC·HCl, 0.0645 g (0.56 mmol) NHS were mixed with Phe-Gly-CS,[10] adding 0.1 mol·L^{-1} HCl to adjust PH at 6, the reation temperature at 25 °C under magnetic stirring for 48 h. The products were purified by dialysis against deionised water for 3 days, and then freeze-dried. White sponge solid was obtained. IR(KBr) : 3384 cm^{-1} (*-OH* and *N-H*), 1653 cm^{-1} (*C=O* of *-CO*N*H*), 1558 cm^{-1} (*C=C* of benzene ring), 1397 cm^{-1} (*O-H*), 900 cm^{-1} (pyran ring of CS and LA); ^{1}H NMR (F_3COOD-DMSO-d_6, 20) d/ppm: 2.009 (N*H*), 2.78~2.86 (C_6H_6CH2), 3.044 (C2*H* of CS and C4*H* of LA), 4.77~4.86 ($C_6H_6CH_2CH$), 5.26 (C1*H* and N*H2* of CS), 7.10~7.30 (benzene ring), 7.71 (CON*H* of LA), 7.79 (CON*H* of dipeptide), 10.19 (COO*H* of dipeptide).

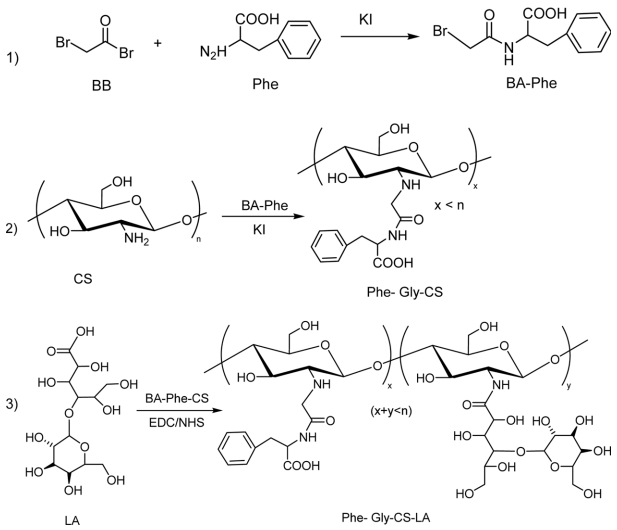
RESULTS AND DISCUSSIONS

Synthesis and characterization of Phe-Gly-CS-LA

The synthesis procedure of Phe-Gly-CS-LA is shown in *Scheme* 1.

In the last step, EDC which is the polymer-supported carbodiimide and NHS that is to synthesize an NHS-activated acid were added for promoting amide linkege formation. EDC is added as a coupling reagent to form a highly unstable activated acid intermediate, NHS reacts to form a less labile activated acid as SuO-. In Thermaodynamics, amide is more stable than ester, so the product can be smoothly obtained under the reaction condition. The structure of Phe-Gly-CS-LA was characterized by IR spectra.

The FTIR spectra of Phe-Gly-CS-LA has similar FTIR spectra with Phe-Gly-CS. While the peak at 1598 cm^{-1} of N-H bending was prominently weaker than that of Phe-Gly-CS, which provided the evidence of loss of C2-amino group of CS. Another evidence for the formation of Phe-Gly-CS-LA was based on the two peaks at 1397 and 1072 cm^{-1}, corresponding to -OH, its intensity was prominently enhanced due to increase of -OH group of LA. The chemical structure of Phe-Gly-CS-LA was further determined by ^{1}H NMR spectroscopy (*Fig.* 1).



***Scheme* 1.** Synthesis of Phe-Gly-CS-LA.

The peaks at 10.19 ppm and 7.10~7.30 ppm were assigned to the COO*H* of depeptide and *H* of benzene ring, which efficiently comfirmed that BA-Phe grafted CS. The peak at 4.97 ppm was due to C1*H* of LA pyran ring, respectively. Its peak is in the low field compared with other *H* of LA pyran ring, as the oxygen atoms around, the electron screening is weaker. From the FTIR spectra and ^{1}H NMR spectra of Phe-Gly-CS-LA, it can be concluded the successful synthesis of Phe-Gly-CS-LA.

Synthesis and characterization of Phe-Gly-CS

KI was used as the catalyst in this step for preparing BA-Phe and Phe-Gly-CS. It was desired to displace Br^- with I^-, improve the reaction rate. The structure of BA-Phe and Phe-Gly-CS were also characterized by IR.

Compared with the IR spectra of CS and the spectra of Phe-Gly-CS, the spectra of Phe-Gly-CS display new important absorption speaks at 1641, 751 and 703 cm^{-1}, which were assigned to the acetyl and benzene ring of BA-Phe. This shift could effectively prove that BA-Phe was grafted onto C2-amino group of CS.

Synthesis and characterization of BA-Phe

The preparation of BA-Phe is crucial for the following

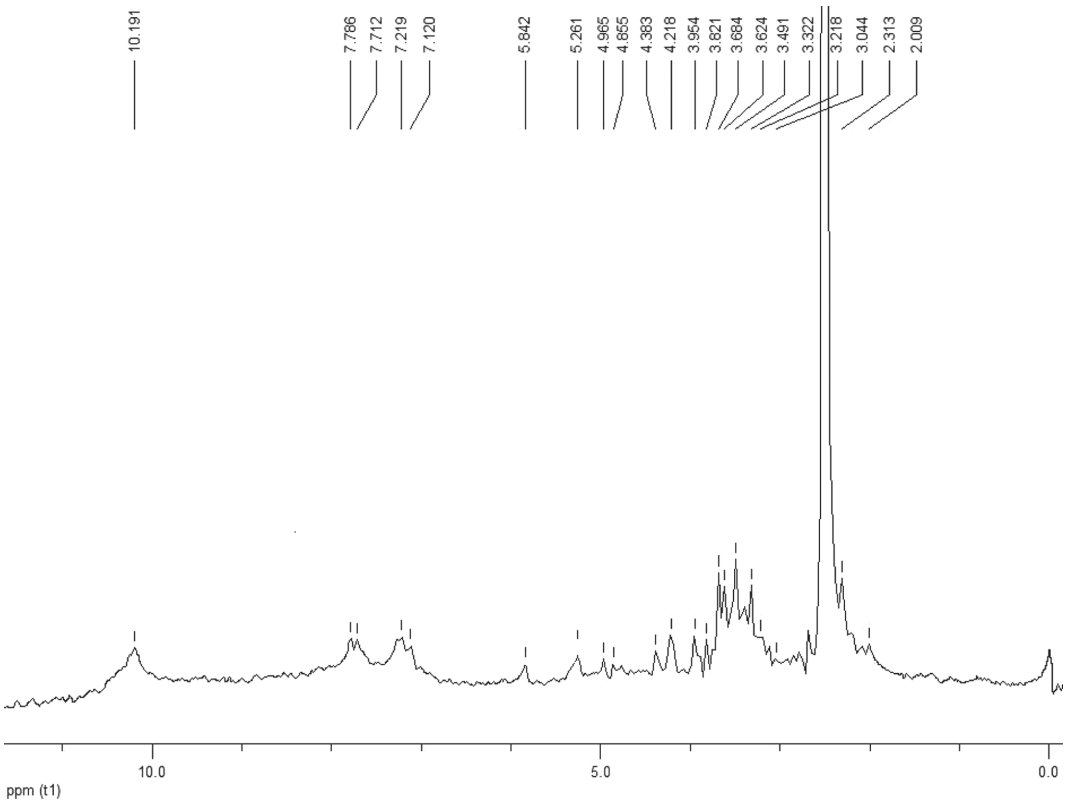


***Fig.* 1.** ^{1}H NMR spectra of Phe-Gly-CS-LA in F_3COOD-DMSO-d_6 at 20 °C.

steps in the whole process. In this study the effects of some reaction conditions such as temperature, reaction time and PH value for synthesis of BA-Phe were optimized. The results were analyzed as described below.

Effect of reaction temperature

The effect of temperature was studied by changing the reaction temperature from -10 to 8 °C and keeping the reaction time for 1.5 h, PH at 11 and other reaction conditions constant (*Table* 1).

Table 1 shows that the yield of reaction increased as the temperature rose from -10 to -4 °C, then, decreased as the temperature rose from 1 to 8 °C. The reaction occurred between bromoacetyl bromide and phenylalanine via an amide linkage is an exothermic reaction. So low temperature is good for reaction and restraining the hydrolysis of bromoacetyl bromide. However, when the temperature is below -7 °C, the molecular motion slowed down, collisions between molecules also reduced, which led to a decrease in yield.

***Table* 2.** The effect of reaction time on yield of reaction

Number	1	2	3	4
Drop time (h)	0.5	1.0	1.5	2.0
Stir time (h)	0.5	0.5	1.0	1.5
Yield (%)	44.5	78.8	79.2	79.6

Effect of reaction time

The effect of reaction time was studied by changing the time from 1.5 to 3.5 h and keeping the temperature at -4 °C, PH at 11 and other reaction conditions constant (*Table* 2).

From *Table* 2, it is clearly found that the yield increased sharply as the reaction time was extended to 1.5 h. While after 1.5 h, the yield efficiency increased very slowly.

***Table* 1.** The effect of temperature on yield of reaction

Number	1	2	3	4	5	6
Temperature/°C	7~8	3~4	0~1	-3~-4	-7<-8	<-10
Yield/%	33.4	58.7	74.3	78.2	63.7	/

***Table* 3.** The effect of pH value on yield of reaction

Number	1	2	3	4
pH	8~9	10~11	12~13	14
Yield (%)	40.5	78.6	77.4	64.2

Effect of pH

In this step, HBr was prepared as by-product. In order to make the reaction carry out completely and improve productivity, the reaction should be under alkaline conditions. The effect of pH was studied while keeping the temperature at -4 ºC, reaction time of 1.5 h and the other reaction conditions constant (*Table* 3).

Table 3 shows the yield of reaction was increased as the increase of PH up to 10-11, then, the yield was decreased as the increase of PH over 11. The decrease in the reaction yield over pH=11 could be explained by the nucleophilic reaction of -OH and -NH_2 with *C*=O of bromoacetyl bromide simultaneously. In a similar study, the observed lower reaction yield at PH=8-9 was attributed to the neutralization reaction of HBr can not be fully completed under the weak base condition.

Characterization of BA-Phe

BA-Phe was prepared by Phe and bromoacetyl bromide via an amide linkage, which is a significant feature compared with Phe. So the reaction happened or not can be decided by its absorption peak in IR spectra. The spectrum of Phe presents the following bands: N-H stretching vibration at 3297 cm^{-1}, O-H and N-H stretching vibration (hydrogen bond) at 3068 cm^{-1}, C-H stretching vibration at 2953 cm^{-1}, -C=O at 1621.79 cm^{-1}, benzene ring stretching vibration at 1585 and 1499 cm^{-1}, C-N and -NH_3^+ stretching vibration at 1213 cm^{-1}, N-H bending vibration at 853 cm^{-1}, C-H axial deformation of benzene ring at 743 and 698 cm^{-1}. In comparison to the Phe IR spectrum, The BA-Phe spectra display a new absorption peak at 637, 612, 586 cm^{-1}, respectively, corresponding to the C-Br tra and cis stretching vibration. The interpretation of the -C=O (NH-*C*=*O*) stretching is at 1621 cm^{-1}, the -C=O (-*CO*OH) stretching is at 1720 cm^{-1}, the O-H (-*CO*OH) stretching is at 1245 and 3550 cm^{-1}, the amide N-H bending region is not trivial contribute to bands in this region. The molecular weight of BA-Phe is 286.12, we measured $[M+Na]^+$ =308.1 by MS (*Fig.* 2).

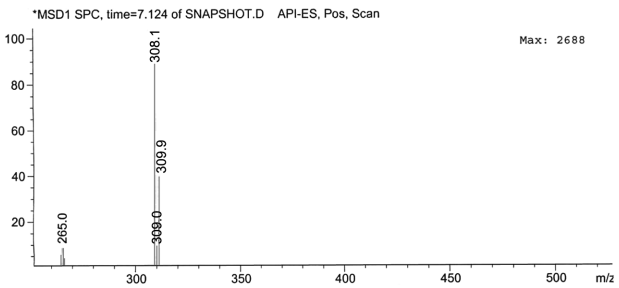


***Fig.* 2.** MS spectra of BA-Phe.

From all above, we can conclude that the produce is as originally desired.

CONCLUSION

We successfully prepared BA-Phe as the key intermediate, then grafted it onto CS, and obtained Phe-BA-CS. The lactobionic acid (LA) was grafted the Phe-Gly-CS and the Phe-Gly-CS-LA was obtained. The product were characterized with IR, MS and ^{1}H NMR. The preparing condition of BA-Phe was optimized, involving the pH 10-11, the temperature -4 ºC, the reaction time 1.5 h and the yield is 78.8%.

Acknowledgements. This work was supported by the National Natural Science Foundation of China (No. 20775010), the Natural Science Foundation of Hunan Province of China (06JJ2038) and Graduate Innovation Fund of Hunan Province of China.

REFERENCES

1. Sui, W. P.; Chen, G. H.; Gao, X. C.; Shang, C. Q.; Sun, M. K. *Chem. J. Chinese Univ.* **2001**, *22*, 133.
2. Jia, J. T.; Dong, C. D.; Zhang, W. L.; Cui, Y. X.; Liu, J. P. *Pharmaceutical and Biomedical Analysis* **2011**, *55*, 342.
3. Lin, W. J.; Chen, T. D.; Liu, C. W. *Polymer* **2009**, *50*, 4166.
4. (a) Kumar, M. N.; Muzzarelli, R.A.; Muzzarelli, C.; Sashiwa, H.; Domb, A. J. *Chem. Rev.* **2004**, *104*, 6017. (b) Patale, R. L.; Patravale, V. B. *Carbohydr. Polym*. **2011**, *85*, 105.
5. (a) Knapczyk, L. K. J.; Krzck, J.; Brzeski, M.; Nirnberg, E.; Schenk, D.; Struszcyk, H. Chitin and Chitosan: Sources, Chemistry, Biochemistry, Physical Properties and Applications; In *Requirements of Chitosan for Pharmaceutical and Biomedical Applications*; G. Skak-Braek, T. Anthonsen, P. Sandford, Eds.; Elsevier: London, 1989, p 657. (b) Illum, L. *Pharm. Res*. **1998**, *15*, 1326. (c) Felt, O.; Buri, P.; Gurny, R. *Drug. Dev. Ind. Pharm*. **1998**, *24*, 979. (d) Bhattarai, N.; Gunn, J.; Zhang, M. Q. *Advanced Drug Delivery Reviews* **2010**, *62*, 83. (e) Lao, S. B.; Zhang, Z. X.; Xu, H. H.; Jiang, G. B. *Carbohydr. Polym*. **2010**, *82*, 1136.
6. (a) Yoo, H. S.; Lee, J. E.; Chung, H.; Kwon, I. C.; Jeong.

S. Y. *J. Control Release* **2005**, *103*, 235. (b) Amidi, M.; Romeijn, S. G.; Borchard, G.; Junginger, H. E.; Hennink, W.E.; Jiskoot, W. *J. Control Release* **2006**, *111*, 107. (c) Kim, J. H.; Kim, Y. S.; Park, K.; Lee, S.; Nam, H. Y.; Min, K. H.; Jo, H. G.; Park, J. H.; Choi, K.; Jeong, S. Y.; Park, R. W.; Kim, I. S.; Kim, K.; Kwon, I. C. *Control Release* **2008**, *127*, 41.

7. (a) Overington, J. P.; Al-Lazikani, B.; Hopkins, A. L. *Drug Discov.* **2006**, *5*, 993. (b) Park, J. H.; Saravanakumar, G.; Kim, K.; Kwon, I. C. *Advanced Drug Delivery Reviews* **2010**, *62*, 28.
8. Li, M. L.; Xu, Y. J.; Jiang, L. Q.; Chen, L. G. *Chemistry* **2006**, *3*, 179.
9. (a) Batista, M. K. S.; Pinto, L. F.; Gomes, C. A. R.; Gomes, P. *Carbohydr. Polym.* **2006**, *64*, 299. (b) Gomes, P.; Gomes, C. A. R.; Batista, M. K. S.; Pinto, L. F. P.; Silva, A. P. *Carbohydr. Polym.* **2008**, *71*, 54.
10. (a) Park, J. H.; Cho, Y. W.; Chung, H.; Kwon, I. C.; Jeong, S. Y. *Biomacromolecules* **2003**, *4*, 1087. (b) Sun, L.; Dai, J. H.; Baker, G. L.; Bruening, M. L. *Chem. Mater.* **2006**, *18*, 4033.