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Polymeric asymmetric reducing agents: preparation and reducing performance of chitosan/dihydronicotinamide conjugates having L- and D-phenylalanine spacer arms

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Abstract

Conjugates composed of chitin and the dihydronicotinamide group having L- and D-phenylalanine spacer arms have been prepared and evaluated as polymer-supported asymmetric reducing agents. Though the coupling reaction of *N*-nicotinoyl-L- or D-phenylalanine with chitosan resulted in poor substitution, the reaction with trityl-chitosan was efficient in solution to attain high substitution degrees. The derivatives were quaternized and reduced to generate the dihydronicotinamide structure. Reduction of ethyl benzoylformate with the resulting conjugates having L-phenylalanine and D-phenylalanine spacer arms produced (−)-excess and (+)-excess ethyl mandelate, respectively, indicating that the chiral selectivity can be controlled by the molecular structure of the spacer arm to synthesize a target enantiomer. The polymeric reducing agents could be regenerated after reaction to allow repeated use and the reducing performance proved to remain in the same level in four consecutive runs.

Keywords: Chitin; Asymmetric reduction; NADH; Polymeric reagent; Spacer arms

1. Introduction

Reagents supported on insoluble polymer matrices are often useful for carrying out synthetic reactions efficiently, since they enable separation and repeated use in a simple manner. One of the most attractive biological reagents to immobilize on polymers is NADH, a coenzyme in the reduction in biological systems. NADH has a dihydronicotinamide group as an active site and there have been many reports on the low molecular weight NADH models as reducing agents and high asymmetric selectivity was achieved (Ohno, Ikeguchi, Kimura & Oka, 1979; Seki, Baba, Oda & Inouye, 1981). Polymer-supported dihydronicotinamide derivatives are interesting in view of their practical use (Eling, Challa & Pandit, 1983; Eling, Hoogsteen, Challa & Pandit, 1984; Eling, Challa & Pandit, 1985; Endo, Takada & Okawara, 1983; Ito, Abe & Senoh, 1987; Tintillier, Dupas, Bourguignon & Quéguiner, 1986). Asymmetric selectivity is, however, known to be low on polymeric supports and the enantiomeric excess (ee) values are less than 10% (Shinkai, Tsuji, Sone & Manabe, 1981), except a recent achievement of 56% with a Merrifield Resin/dihydronicotinamide derivative (Losset, Dupas, Bourguignon & Quéguiner, 1989).

Among the polymer candidates for supporting active species, chitosan is considered promising because of the presence of free amino groups. Furthermore, the rigid chiral architecture of the polysaccharide would be advantageous for favorable influence on asymmetric interaction with substrates. In order to elucidate the influence of the chiral field of chitin on the reducing performance of the dihydronicotinamide moiety, we have prepared various chitin/dihydronicotinamide conjugates. Reduction of ethyl benzoylformate with the conjugates revealed that direct linking of the dihydronicotinamide group to the amino group of chitosan resulted in high asymmetric selectivity with low chemical yield (Kurita, Koyama, Murakami, Yoshida & Chau, 1986). Introduction of oligo(L-alanine) spacer arms increased the chemical yield but reduced the ee value (Kurita, Iwawaki, Ishii & Nishimura, 1992). As a spacer, L-alanine was appropriate for improving chemical yield without sacrificing the asymmetric selectivity much (Nishiyama, Yoshida, Mori, Ishii & Kurita, 1998b).

These results suggest the usefulness of amino acids as spacer arms between the chitin backbone and the active site. As a consequence of these studies, structural factors of spacer arms have been further examined using L- and

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Table 1
Coupling reaction of *N*-nicotinoyl-phenylalanine with chitosan or trityl-chitosan

Chitosan	Nicotinoyl-phenylalanine/ $-NH_2$ (mol mol^{-1})	Spacer	ds[a]
Chitosan	3.0	L-Phe	0.18[b]
Chitosan	3.0	D-Phe	0.17[c]
Trityl-chitosan	1.0	L-Phe	0.66[d]
Trityl-chitosan	1.0	D-Phe	0.69[e]
Trityl-chitosan	1.5	L-Phe	0.81[f]
Trityl-chitosan	1.5	D-Phe	0.91[g]

[a] Degree of substitution calculated from the C/N value of elemental analysis.
[b] Calcd for $(C_{21}H_{13}N_3O_6)_{0.18}(C_6H_{11}NO_4)_{0.82}\cdot 1.4H_2O$: C, 45.08; H, 6.94; N, 8.22. Found: C, 45.00; H, 7.19; N, 8.21.
[c] Calcd for $(C_{21}H_{13}N_3O_6)_{0.17}(C_6H_{11}NO_4)_{0.83}\cdot H_2O$: C, 46.24; H, 6.83; N, 8.45. Found: C, 46.17; H, 7.11; N, 8.45.
[d] Calcd for $(C_{40}H_{37}N_3O_6)_{0.66}(C_{25}H_{25}NO_4)_{0.34}\cdot 1.8H_2O$: C, 68.94; H, 6.16; N, 5.37. Found: C, 68.97; H, 6.16; N, 5.35.
[e] Calcd for $(C_{40}H_{37}N_3O_6)_{0.69}(C_{25}H_{25}NO_4)_{0.31}\cdot 1.8H_2O$: C, 64.46; H, 6.47; N, 5.06. Found: C, 64.46; H, 6.47; N, 5.06.
[f] Calcd for $(C_{40}H_{37}N_3O_6)_{0.81}(C_{25}H_{25}NO_4)_{0.19}\cdot 1.9H_2O$: C, 69.49; H, 6.04; N, 5.72. Found: C, 69.49; H, 5.82; N, 5.72.
[g] Calcd for $(C_{40}H_{37}N_3O_6)_{0.91}(C_{25}H_{25}NO_4)_{0.09}\cdot 2.8H_2O$: C, 67.91; H, 6.12; N, 5.77. Found: C, 67.86; H, 5.85; N, 5.74.

D-phenylalanines, which would be suitable to discuss the possibility of chiral recognition by spacer arms and the influence of increased hydrophobicity on accommodation of the substrate.

2. Experimental

2.1. General

IR spectra were recorded on a JASCO IRA-700 instrument. ^{1}H NMR spectra were taken with a JEOL JNM-LA400D. Optical rotation was measured in *N*,*N*-dimethylformamide (DMF) or in ethanol with a JASCO DIP-370 digital polarimeter at room temperature. Elemental analysis was performed with a Perkin Elmer 2400. The content of free amino group was determined by conductometric titration with a TOA conductivity meter CM-40S. TLC was performed on Merck silica gel 60F254 plates. Solvents were purified in the usual way and stored over molecular sieves.

2.2. Chitosan

Chitin isolated from shrimp shells was pulverized and treated with 40% aqueous sodium hydroxide at 110°C for 4 h three times to give chitosan as an almost colorless powdery material. The degree of deacetylation was 1.0 as determined by conductometry.

2.3. Preparation of 6-O-trityl-chitosan

Trityl-chitosan was prepared according to the previously reported method based on *N*-phthaloyl-chitosan (Nishimura, Kohgo, Kurita & Kuzuhara, 1991). It was obtained as a white powdery material; the overall yield from chitosan was 61% and the degree of substitution (ds) was 1.0.

2.4. Nicotinic acid N-hydroxysuccinimide ester

The ester formation between nicotinic acid and *N*-hydroxysuccinimide was carried out in a manner similar to the reported method where *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl) was used (Cohen, Stoddard & Koshland, 1997).

To a solution of 12.3 g (0.10 mol) of nicotinic acid in 200 ml of pyridine were added 21.1 g (0.10 mol) of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 11.5 g (0.10 mol) of *N*-hydroxysuccinimide and the mixture was stirred at room temperature. After 20 h, TLC confirmed the completion of the reaction and the mixture was filtered. The filtrate was concentrated under reduced pressure to give a white solid. The solid was dissolved in 300 ml of chloroform and the solution was washed with 50 ml each of deionized water three times. The chloroform solution was dried over sodium sulfate and evaporated under reduced pressure to dryness. The resulting white solid was recrystallized from ethanol to give 16.4 g (75% yield) of colorless needles; m.p. 133–135°C. IR (KBr): ν 1796, 1773, 1740, and 1727 (C=O), 1590 (arom), 1205 (C–O–C), and 719 cm^{-1} (Pyr). ^{1}H NMR ($CDCl_3$): δ 2.90 (s, 4H, CH_2), 7.47 (m, 1H, Pyr 5-H), 8.36 (d, 1H, Pyr 4-H), 8.87 (d, 1H, Pyr 6-H) and 9.30 ppm (d, 1H, Pyr 2-H).

2.5. N-Nicotinoyl-L-phenylalanine

The succinimido ester of nicotinic acid obtained above (5.0 g, 22.7 mmol) was dissolved in 250 ml of 1,2-dimethoxyethane and the solution was cooled in an ice-water bath. A solution of 3.75 g (22.7 mmol) of L-phenylalanine in 100 ml of 10% aqueous sodium carbonate was added dropwise over a period of 30 min and the mixture was stirred at 0°C for 2 h and then at room temperature for 14 h. It was filtered and the filtrate was concentrated to ca. 100 ml under reduced pressure. The solution was acidified to pH 4 with 10% citric acid and extracted with 500 ml of

ethyl acetate. The extract was dried with sodium sulfate and evaporated under reduced pressure. The resulting white solid was recrystallized from ethanol to give colorless small crystals. The yield was 3.45 g (56%); m.p. 175–178°C (lit. 176–177°C (Bixler & Niemann, 1958)).

N-Nicotinoyl-D-phenylalanine was synthesized in the same way from D-phenylalanine. The yield was 60%; m.p. 174–175°C; R_f 0.21 (chloroform/methanol/acetic acid = 90:8:2); $[\alpha]_D = +72.5°$ (*c* 10.0, DMF). The IR and NMR spectra were identical with those of the L-derivative. IR (KBr): ν 3334 (NH), 1731 (COOH), 1640 (amide I), 1599 (arom), and 1537 cm^{-1} (amide II). ^1H NMR (pyridine-d_5): δ 3.50 (m, 1H, CH_2), 3.73 (m, 1H, CH_2), 5.64 (m, 1H, CH), 7.22–7.32 (m, 5H, Ph), 7.50 (m, 1H, Pyr 5-H), 8.35 (d, $J = 8.0$ Hz, 1H, Pyr 4-H), 8.72 (d, $J = 4.6$ Hz, 1H, Pyr 6-H), 9.53 (s, 1H, Pyr 2-H), and 9.70 ppm (d, 1H, NH).

Anal. Calcd for $C_{15}H_{14}N_20_3$: C, 66.77; H, 5.69; N, 10.31. Found: C, 66.22; H, 5.26; N, 10.30.

2.6. Coupling of *N*-nicotinoyl-L-phenylalanine with chitosan

Chitosan (degree of deacetylation 1.0, 1.00 g, 6.2 mmol glucosamine units) was dispersed in 50 ml of dimethyl sulfoxide (DMSO) and 5.03 g (18.6 mmol) of *N*-nicotinoyl-L-phenylalanine, 3.84 g (18.6 mmol) of DCC, and 2.85 g (18.6 mmol) of *N*-hydroxybenzotriazole monohydrate (HOBt·H_2O) were added. The mixture was stirred at room temperature for 24 h and filtered. The solid was washed with acetone. It was further washed thoroughly with ethanol and then with acetone in a Soxhlet extractor. The product was obtained as a pale tan powdery material; yield 1.15 g. The ds was 0.18 as calculated from the C/N value of the elemental analysis data (see footnote of Table 1). IR (KBr): ν 1650 (amide I), 1594 (arom), 1530 (amide II), and 1150–1000 cm^{-1} (pyranose).

With *N*-nicotinoyl-D-phenylalanine, the coupling reaction was conducted by the same method to prepare the product; yield 1.19 g. The ds was calculated to be 0.17 from the elemental analysis data (see footnote of Table 1). The IR spectrum was identical with that of the L-derivative.

2.7. Coupling of *N*-nicotinoyl-L-phenylalanine with 6-*O*-trityl-chitosan

To a solution of 4.00 g (9.9 mmol of pyranose units) of trityl-chitosan in 200 ml of *N*,*N*-dimethylacetamide (DMAc) were added a solution of 2.68 g (9.9 mmol) of *N*-nicotinoyl-L-phenylalanine, 1.52 g (9.9 mmol) of HOBt·H_2O, and 2.18 g (11 mmol) of EDC·HCl in 80 ml of dichloromethane. The mixture was stirred at room temperature for 24 h. It was cooled in an ice-water bath and 200 ml of deionized water was added. The precipitate was collected on a filter, washed with acetone and then with methanol, and dried to give 4.32 g of a pale tan powdery material. The ds was 0.66 as determined from the C/N value of the elemental analysis data (see footnote of Table 1). IR (KBr): ν 3058 (arom CH), 1656 (amide I), 1593 (arom), 1519 (amide II), 1150–1000 (pyranose), 744 (arom), and 698 cm^{-1} (arom).

N-Nicotinoyl-D-phenylalanine was coupled with trityl-chitosan in the same way to give the corresponding derivative with ds 0.69. The IR spectrum was identical to that of the L-derivative.

With 1.5 equiv. of the reagents to the amino group of trityl-chitosan, higher substitution was achieved and the ds values were 0.81 and 0.91 for L- and D-phenylalanine derivatives, respectively. The subsequent reactions were performed with these products.

2.8. Acetylation of the free amino groups

N-(*N*-Nicotinoyl-L-phenylalanyl)-6-*O*-trityl-chitosan (3.70 g) obtained above was dispersed in 100 ml of methanol and 5.3 ml of acetic anhydride was added to acetylate the remaining free amino groups. The mixture was stirred at room temperature for 24 h and poured into 500 ml of deionized water. The solid was filtered, washed with methanol and dried to give 3.70 g of a pale tan powder.

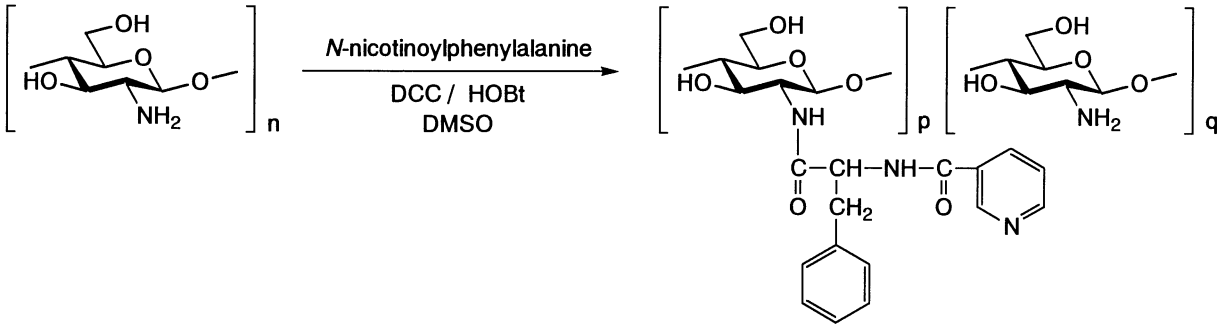
The possible *O*-acetyl groups after acetylation were removed as follows. The product was pulverized and dispersed in 200 ml of dry methanol and 0.5 g of sodium was added. The mixture was stirred in nitrogen at room temperature for 24 h and poured into 500 ml of deionized water. The solid was filtered, washed with acetone and dried to give 3.67 g of the acetylated product; yield 3.67 g. Conductometric titration indicated the absence of free amino groups and the ninhydrin test was negative. IR (KBr): ν 3060 (arom CH), 1660 (amide I), 1592 (arom), 1520 (amide II), 1150–1000 (pyranose), 743 (arom), and 698 cm^{-1} (arom).

The corresponding D-phenylalanine derivative was acetylated with acetic anhydride and treated with methoxide in the same way. The IR spectrum was identical to that of the L-phenylalanine derivative. Conductometric titration and ninhydrin test showed no free amino groups.

2.9. Detritylation

To 2.90 g of *N*-(*N*-nicotinoyl-L-phenylalanyl)-6-*O*-trityl-chitosan was added 35 ml of dichloroacetic acid and the mixture was stirred at room temperature for 2 h. The resulting solution was poured into water to precipitate the product. It was filtered, washed with methanol and dried to give 1.71 g of *N*-(*N*-nicotinoyl-L-phenylalanyl)-chitosan as a pale tan powdery material. IR (KBr): ν 1652 (amide I), 1594 (arom), 1541 (amide II), 1150–1000 (pyranose) and 701 cm^{-1} (arom).

Detritylation of the D-phenylalanine derivative was performed by the same method. Starting from 1.86 g of *N*-(*N*-nicotinoyl-D-phenylalanyl)-6-*O*-trityl-chitosan, 0.93 g of *N*-(*N*-nicotinoyl-D-phenylalanyl)-chitosan was obtained.



Scheme 1.

2.10. Quaternization of the nicotinoyl group

To a dispersion of 2.15 g of *N*-(*N*-nicotinoyl-L-phenylalanyl)-chitosan in 100 ml of DMF was added 1.98 g (3 equiv. to pyranose unit) of benzyl chloride and the mixture was heated at 80°C for 48 h with stirring. After cooling to room temperature, 50 ml of methanol was added. The solid was filtered, washed with methanol and dried to give 1.95 g of a pale tan powdery material. IR (KBr): ν 3070 (arom CH), 1664 (amide I), 1546 (amide II), 1150–1000 (pyranose) and 700 cm^{-1} (arom).

The D-isomer, *N*-(*N*-nicotinoyl-D-phenylalanyl)-chitosan (0.56 g), was quaternized by the same method to yield 0.43 g of the product.

2.11. Formation of dihydronicotinamide structure

The quaternized product (1.00 g) prepared above was pulverized and dispersed in 30 ml of DMF. To the dispersion were added a solution of 0.30 g (2.78 mmol) of sodium hydrosulfite in 5 ml of water and a solution of 0.30 g (2.78 mmol) of potassium carbonate in 5 ml of water. After stirring the mixture at room temperature for 24 h, the reduced product was filtered, washed with deionized water and acetone, and dried to give 0.81 g of the chitin/dihydronicotinamide conjugate as a pale tan powdery material; Beilstein test, negative. The resulting dihydronicotinamide group was determined by oxidation–reduction titration with ferricyanide as reported previously (Kurita et al., 1992), and the content was calculated to be 1.37 meq g^{-1}. IR (KBr): ν 1650 (amide I), 1555 (amide II) and 1150–1000 cm^{-1} (pyranose).

Starting from 1.07 g of the quaternized D-isomer, reduction under the same conditions gave 0.80 g of the reduced form. The content of the dihydronicotinamide group was 1.37 meq g^{-1}.

2.12. Reduction of ethyl benzoylformate with the conjugate

The conjugate having L-phenylalanine spacer arms (1.37 meq g^{-1} dihydronicotinamide, 0.50 g) was dispersed in 20 ml of acetonitrile and 0.30 g (2.1 mmol, 3 equiv. to dihydronicotinamide group) of magnesium perchlorate and 0.24 g (2.1 mmol, 3 equiv. to dihydronicotinamide group) of ethyl benzoylformate were added. The mixture was stirred at 40°C for 72 h and filtered to recover 0.43 g of the oxidized conjugate. The filtrate was concentrated under reduced pressure and 10 ml of water was added to the residual light yellow oil. The mixture was extracted with 10 ml each of diethyl ether five times. The combined extract was dried over sodium sulfate and evaporated. The residue was extracted with five 10 ml-portions of dichloromethane and the extract was concentrated under reduced pressure to give 0.21 g of a light yellow oil, which was a mixture of ethyl mandelate and ethyl benzoylformate. The yield of ethyl mandelate determined by ^{1}H NMR spectroscopy (Nishiyama et al., 1998b) was 15.0% based on the amount of the dihydronicotinamide group. $[\alpha]_D$ of the resulting ethyl mandelate was $-13.3°$ in ethanol.

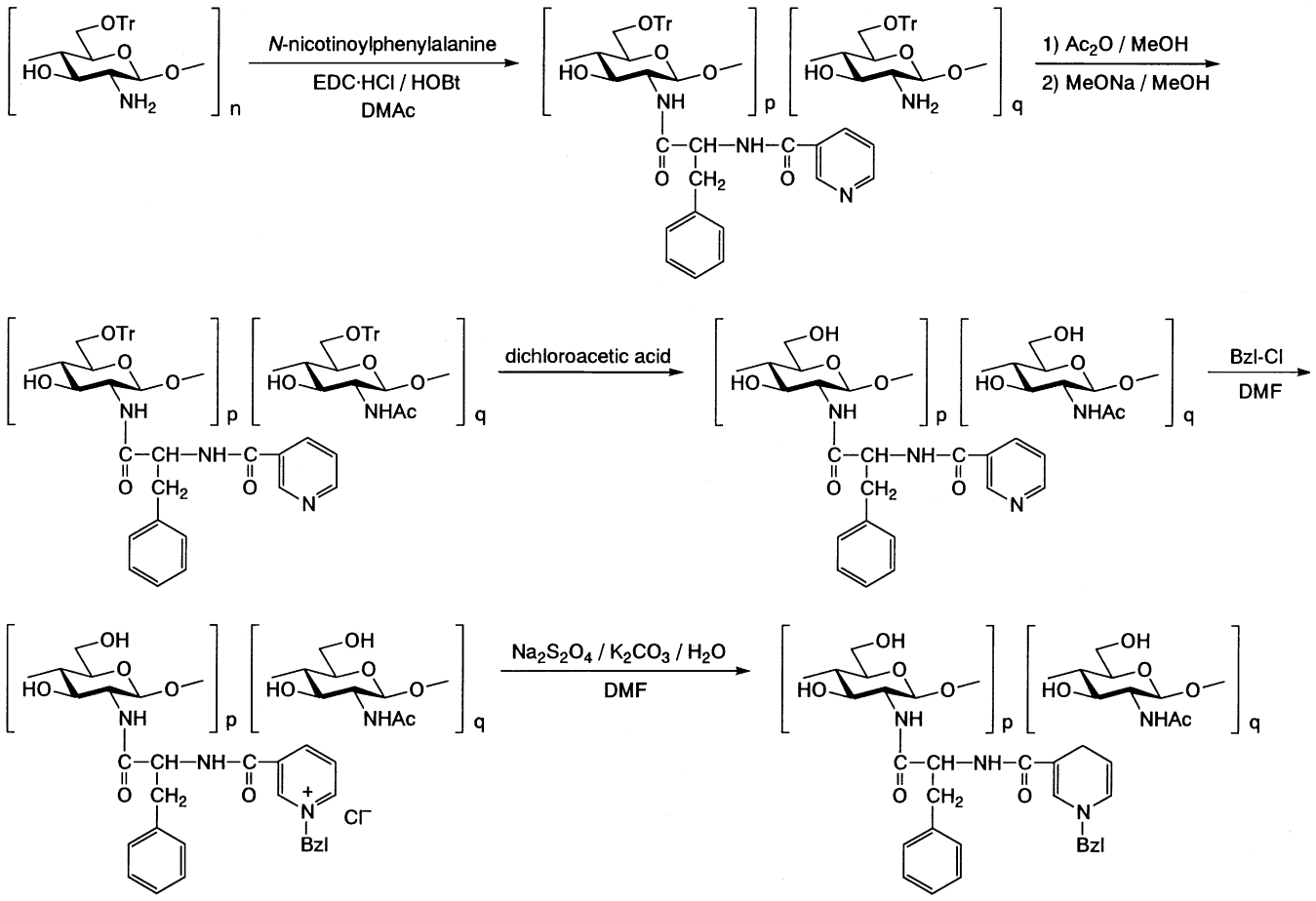
In the reduction of ethyl benzoylformate with the conjugate having D-phenylalanine spacer arms, the yield and $[\alpha]_D$ of ethyl mandelate were 14.0% and $+18.7°$.

2.13. Repeated use of the conjugate

The recovered conjugates after reduction of ethyl benzoylformate were treated with sodium hydrosulfite as given in Section 2.11 to regenerate the dihydronicotinamide structure and they were used again for the reduction of ethyl benzoylformate under the same conditions.

3. Results and discussion

In order to elucidate the influence of spacer arms on the reduction performance, L- and D-phenylalanines were incorporated between the chitin backbone and the NADH active site, 1,4-dihydronicotinamide. Conjugation would be possible either by coupling of nicotinic acid with *N*-phenylalanyl-chitosan or by coupling of *N*-nicotinoyl-phenylalanine with chitosan. The former method, however, will bring about structural ambiguity due to the difficulty in thorough substitutions for the introduction of phenylalanyl groups to chitosan and for the following nicotinoylation at the phenylalanyl residues. In the latter strategy, the resulting product is structurally simple and well defined, being composed of



Scheme 2.

N-(*N*-nicotinoylalanyl)-glucosamine and glucosamine units along the main chain. Chitin/dihydronicotinamide conjugates were thus prepared by the latter method.

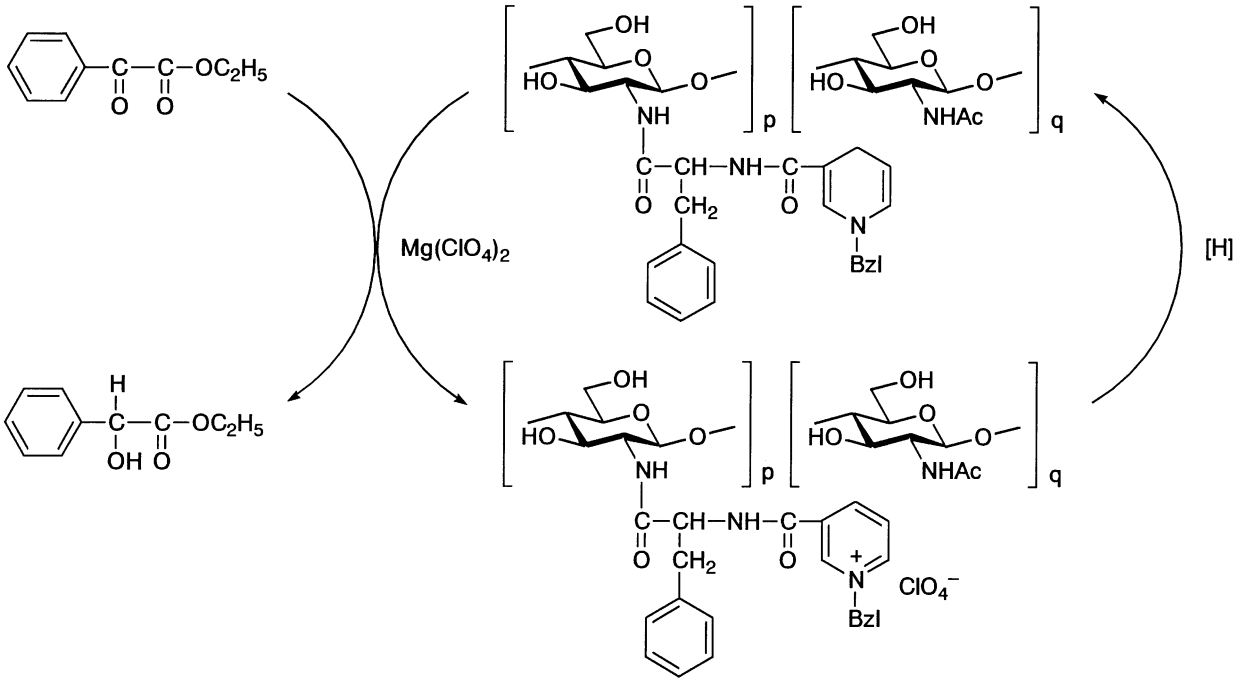
3.1. Conjugation with chitosan

L- and D-Phenylalanines were nicotinoylated with an active ester synthesized from nicotinic acid and *N*-hydroxysuccinimide. The resulting *N*-nicotinoylated phenylalanines were coupled with chitosan in DMSO in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and *N*-hydroxybenzotriazole (HOBt) (Scheme 1). The reaction proceeded under heterogeneous conditions and the ds values were 0.18 and 0.17 for L- and D-derivatives with three-fold excess reagents as listed in Table 1.

The coupling reaction was expected to be much more facile leading to higher substitution degrees when carried out in solution and trityl-chitosan was then used in place of chitosan. Trityl-chitosan is an organosoluble chitosan derivative convenient for chemical modifications at the free amino groups (Nishimura, Miura, Ren, Sato, Yamagoshi, Nishi et al., 1993; Nishimura, Kai, Shinada, Yoshida, Tokura, Kurita et al., 1998; Nishiyama, Yoshikawa, Ohara, Kurita, Hojo, Kamada et al., 2000) and thus the DMAc solution was treated with a dichloromethane solution of *N*-nicotinoylated phenylalanines containing EDC and HOBt. The reaction proceeded efficiently in solution at room temperature to give products having ds values of 0.6–0.7 even with an equivalent amount of *N*-nicotinoylated phenylalanines (Scheme 2). The ds could be increased to 0.8–0.9 with 1.5 equiv. of reagents. Some typical results are included in Table 1. The coupling reaction with trityl-chitosan thus proved superior to that with chitosan in terms of the ds of the products and the subsequent derivatizations were conducted with these products.

The resulting products had some remaining free amino groups, which may interfere with the succeeding reactions resulting in the formation of ambiguous molecular structures. They were thus acetylated with acetic anhydride in methanol and then treated with methoxide to remove possibly formed *O*-acetyl groups. The absence of free amino groups was confirmed by conductometric titration and ninhydrin test.

Trityl groups were then removed with dichloroacetic acid under mild conditions to give *N*-(*N*-nicotinoyl-L- and D-phenylalanyl)-chitosans, which were subsequently quaternized with benzyl chloride and reduced to generate the 1,4-dihydronicotinamide structure (Scheme 2). The Beilstein



Scheme 3.

test supported the absence of chloride ions. The amounts of the dihydronicotinamide group were around 1.3 meq g^{-1}.

3.2. Reduction behavior of the conjugates

The resulting chitosan/dihydronicotinamide conjugates having L- and D-phenylalanine spacer arms were evaluated as polymeric asymmetric reducing agents by virtually the method reported recently (Nishiyama et al., 1998b) as follows. The reduction of ethyl benzoylformate with the same conjugates was conducted in the presence of magnesium perchlorate in acetonitrile under heterogeneous conditions (Scheme 3). After the reaction, the yield of ethyl mandelate was determined by ^{1}H NMR spectroscopy and the optical rotation was measured in ethanol.

As summarized in Table 2, ethyl mandelate was obtained in a similar yield with either L-conjugate or D-conjugate (15.0 and 14.0% based on the dihydronicotinamide). It is noteworthy that the two kinds of ethyl mandelate exhibited opposite optical rotations; L-conjugate favored the formation of (−)-isomer while D-conjugate favored (+)-isomer. Similar asymmetric selectivity was observed with low-molecular weight chiral dihydronicotinamide models (Ohnishi, Numakunai, Kimura & Ohno, 1976). However, some derivatives having L-amino acids gave either (−)- or (+)-mandelate depending on the amino acid (Endo, Hayashi & Okawara, 1977). Furthermore, enantiospecificity is often affected by many factors including solvent, temperature, additives, metal ion and aging time; the configurations could be even opposite for the products obtained at different stages of reduction (Ohno, Kimura, Oka & Ohnishi, 1978). These results suggest rather loose spatial arrangement at the transition state, but our data show that the asymmetric selectivity by polymer-supported reagents can be controlled by the choice of appropriate chiral spacer arms.

When the reduction was carried out with 10 equiv. of ethyl benzoylformate, the chemical yield increased to

Table 2
Reduction of ethyl benzoylformate with chitosan/dihydronicotinamide conjugates (DHN, dihydronicotinamide; EBF, ethyl benzoylformate)

Conjugate		EBF/DHN	Ethyl mandelate		
Spacer	DHN (meq g^{-1})	(mol/mol)[a]	Yield (%)	$[\alpha]_D$ (degree)[b]	ee (%)
L-Phe	1.37	3.0	15.0	−13.3	12.8
D-Phe	1.37	3.0	14.0	+18.7	18.0
L-Phe	1.07	10.0	22.8	−14.7	14.1
D-Phe	1.35	10.0	24.6	+15.5	14.9

[a] Molar ratio in the reduction.
[b] In ethanol. (*R*)-Ethyl mandelate, $[\alpha]_D = -104°$.

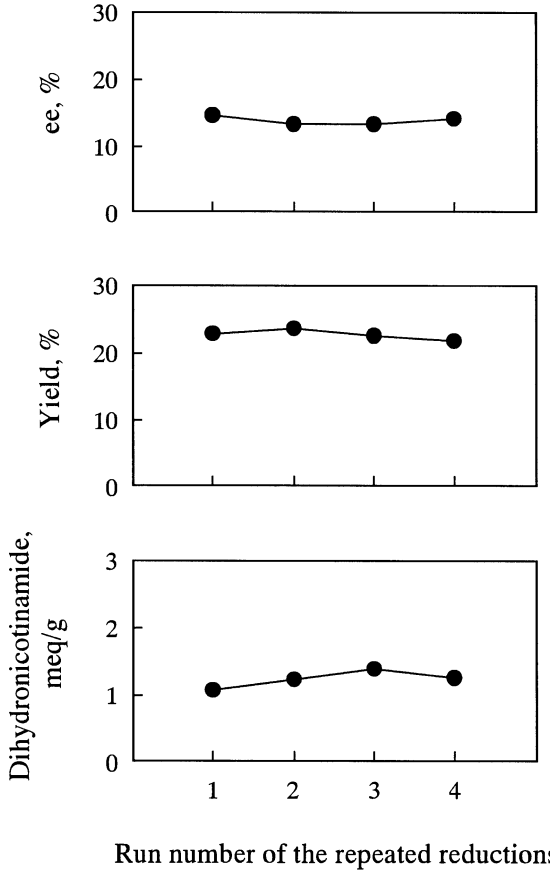


Fig. 1. Reduction profiles of the chitin/dihydronicotinamide conjugate with L-phenylalanine spacer arms in repeated use.

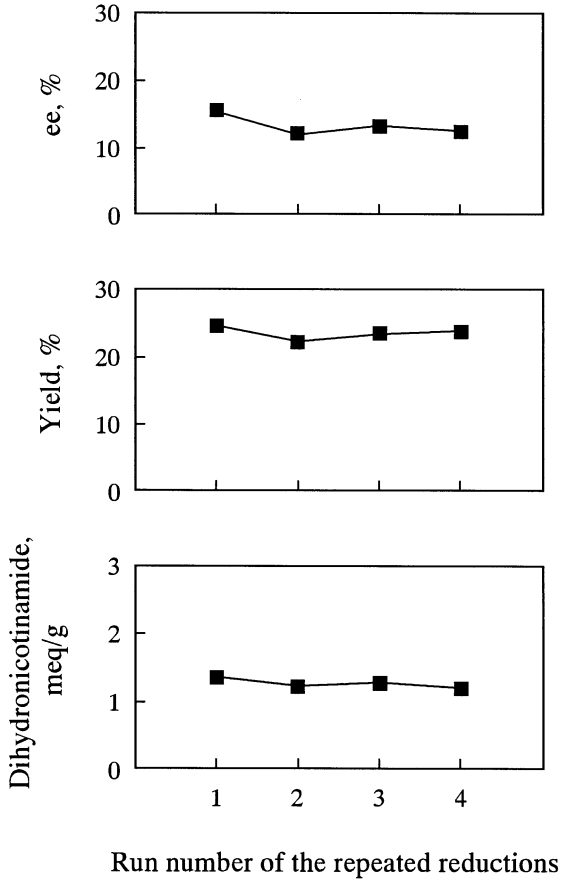


Fig. 2. Reduction profiles of the chitin/dihydronicotinamide conjugate with D-phenylalanine spacer arms in repeated use.

above 20% as included in Table 2. However, the optical rotation values of ethyl mandelate were similar to those with 3 equivalents.

These chemical yields were somewhat lower than those (up to 36%) obtained with chitosan/dihydronicotinamide conjugate having L-alanine spacer arms (Nishiyama et al., 1998b) and this may be attributable to the steric hindrance by the bulky phenyl group on the accommodation of the substrate ethyl benzoylformate.

3.3. Repeated use of the conjugates

Besides the merit of easy separation after reaction, polymer-supported reducing agents have the possibility of repeated use. The recovered conjugates were thus reduced in the same manner and the regenerated conjugates were used for the reduction again (Scheme 3). As shown in Fig. 1 for the L-conjugate in the four consecutive runs with 10 equiv. of ethyl benzoylformate, the dihydronicotinamide group could be quantitatively regenerated and the reducing performance was maintained. The amount of dihydronicotinamide group, chemical yield and ee value were in similar levels, 1.07–1.38 meq g^{-1}, 21.7–23.6% and 13.3–14.6%, respectively. With the D-conjugate, similar results were again obtained as evident in Fig. 2 and the values were 1.20–1.35 meq g^{-1}, 22.3–24.6% and 12.1–15.5%. These values mean that a series of the repeated reactions are surprisingly reproducible taking into account that all the reactions were carried out under heterogeneous conditions in suspension. With 3 equiv. of ethyl benzoylformate, high reproducibility was also confirmed. The high retention of the performance in repeated use in contrast to the decreases observed for conjugates with polystyrene (Eling et al., 1984, 1985) supports the suitability of conjugation on chitosan.

4. Conclusions

Conjugation of chitosan with dihydronicotinamide was successful using *N*-nicotinoyl-phenylalanine and trityl-chitosan as an organosoluble chitosan precursor. The derived chitosan/dihydronicotinamide conjugates having L- or D-phenylalanine spacer arms were confirmed effective as polymeric reducing agents in the reduction of ethyl benzoylformate. Although the chemical yields and ee values were moderate, the two kinds of conjugates afforded reduction products with opposite optical rotations depending

on the configuration of the spacer arms, indicating the important role of the spacer arms in asymmetric recognition. The chiral selectivity can, therefore, be controlled by constructing appropriate molecular environment including spacer arms and polymeric reducing agents with tailored reducing performance would be designed. The remarkably reproducible reduction profiles of the conjugates in repeated use support the high potential as polymeric agents.

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