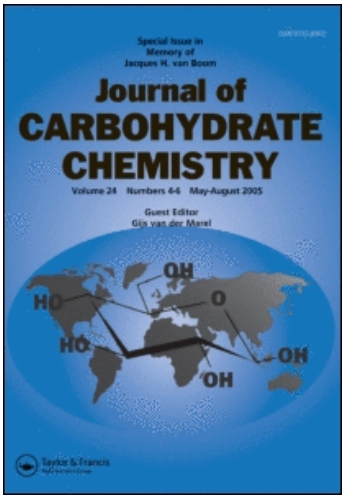

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SYNTHESIS OF A NEW POLYMER CONTAINING URIDINE AND GALACTOSE AS PENDENT GROUPS

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ABSTRACT

A new styrene compound containing a derivatized uridine unit, that is, 2',3'-*O*-isopropylideneuridine 5'-*p*-styrenesulfonate (**1**), was synthesized and polymerized with AIBN as an initiator. Removal of protecting isopropylidene groups from the obtained polymer gave uridine-containing polystyrene. Uridine-containing polystyrene was synthesized also by the polymerization of the deprotected monomer (**2**), which had been prepared by removal of isopropylidene group from **1**. Copolymerization of **1** with a styrene monomer having a galactosyl moiety, that is, *N*-*p*-vinylbenzyl-4-*O*-(β-D-galactopyranosyl)-D-gluconamide[1] (**3**), was carried out in dimethyl sulfoxide. However, the deprotection of the obtained copolymer failed, because the lactonamide portion was severed in the process of deisopropylidenation. On the other hand, the copolymerization of **2** with **3** in dimethylformamide and in water with AIBN as an initiator gave the target copolymer which contained both uridine and galactose residues. Polymers and copolymers were characterized by ^{1}H NMR spectroscopy.

INTRODUCTION

Nucleosides are constituents of biomolecules such as nucleic acids, NAD, ATP, and sugar nucleotides and play important roles in a variety of biochemical reactions. In the biosynthetic reactions of oligosaccharides and polysaccharides, most of donors of glycosylations are sugar nucleotides, of which both the sugar residue and the nucleotide residue must be recognized by glycosyl transferases. The sugar nucleotide generally consists of sugar residue and its corresponding nucleotide residue (UDP-glucose, UDP-galactose, UDP-*N*-acetylglucosamine, UDP-*N*-acetylgalactosamine, GDP-mannose, GDP-fucose, CMP-*N*-acetylneuraminic acid, *etc.*). However, in some exceptional cases,

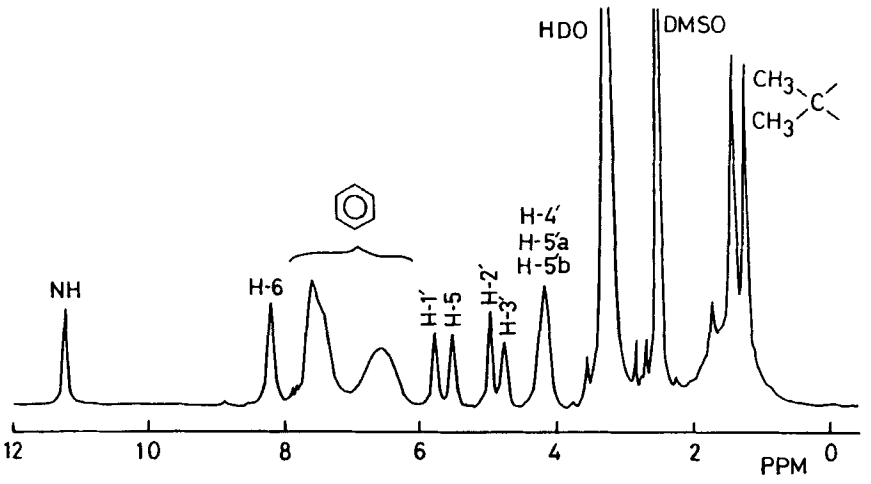

FIG. 1. ^{1}H NMR spectrum of poly(**1**)

the unusual sugar nucleotide such as GDP-glucose is necessary as the substrate for the glycosylation reaction.[2]

It is important to synthesize the functional polymer having the base of nucleotide, which can interact with a lot of biomolecules such as nucleic acids. It has been reported that the synthetic polymers having the base of the nucleotide have important biofunctions.[3] The synthetic poly(9-vinyladenine) recognizes DNA and RNA well,[4] and the polymers of acrylamide and the methacrylamide derivatives of nucleic acid bases interact with natural nucleic acids.[5] Poly(9-vinyladenine-*co*-vinylamine) selectively and efficiently hydrolyzed RNAs under mild conditions,[6] because of the synergism of two kinds of pendent residues.

On the other hand, it has been reported that the sugar-containing polymer is recognized by the receptor on the cell surface and is suitable for the substratum of cell culture.[7]

In this investigation, a styrene monomer having uridine, which contains both uracil and ribose residues, was polymerized to introduce a biological function into the polymer materials. In addition, the uridine-containing monomer was copolymerized with a styrene monomer having galactose residue in order to give a polymeric compound which may be recognized by galactosyl transferase whose substrate is UDP-galactose.

RESULTS AND DISCUSSION

Synthesis of Poly(uridine 5'-*p*-styrenesulfonate). It was reported that the treatment of 2',3'-*O*-isopropylideneuridine with acryloyl chloride in pyridine caused

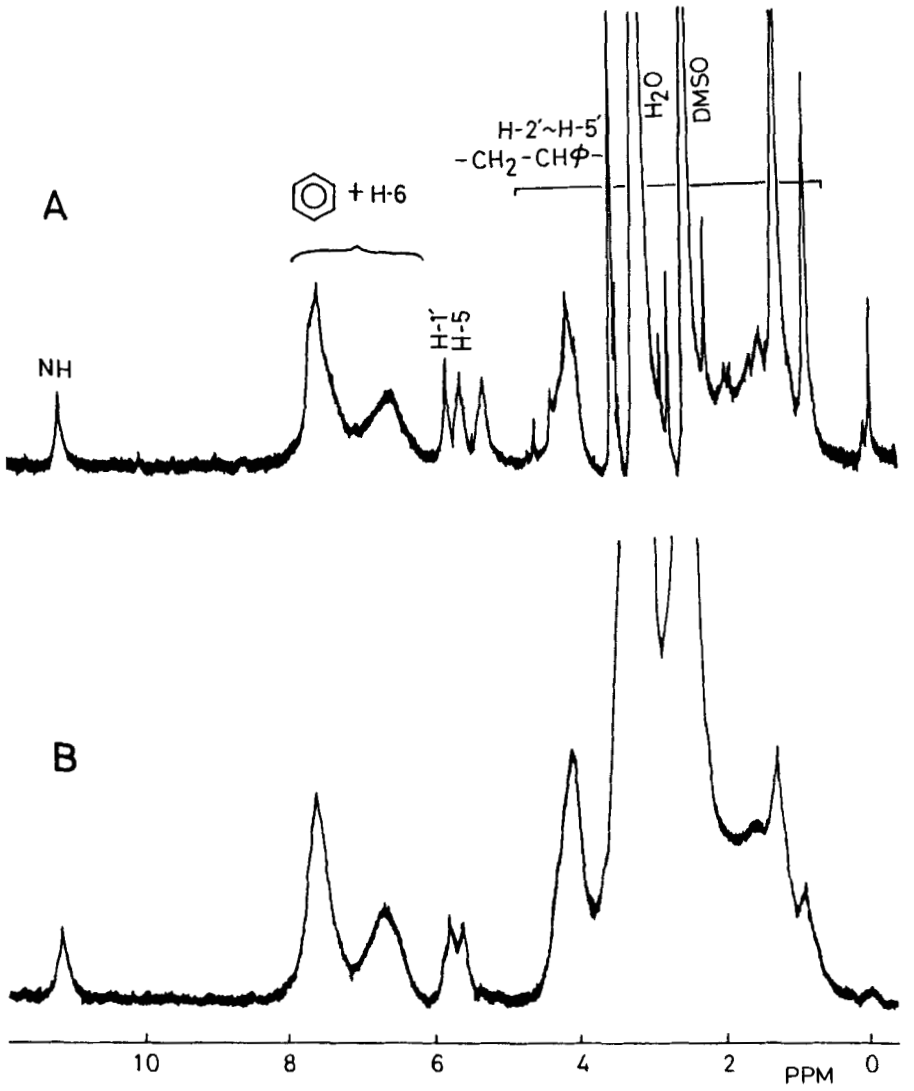

FIG. 2. ^{1}H NMR spectra of (A) deprotected poly(**1**) and (B) poly(**2**).

an addition reaction of pyridine to the double bond,[8] while the reaction of 2',3'-*O*-isopropylideneuridine with acrylic anhydride gave the polymerizable 2',3'-*O*-isopropylideneuridine 5'-acrylate.[9] In this study, 2',3'-*O*-isopropylideneuridine was allowed to react with *p*-styrenesulfonyl chloride at 0° C under high vacuum to give a new polymerizable monomer containing the sulfate group (**1**).

Polymerization of **1** was carried out in *N*,*N*-dimethylformamide (DMF) with 2,2'-azobis(isobutyronitrile) (AIBN) as an initiator at 70° C under high vacuum for 2 days to

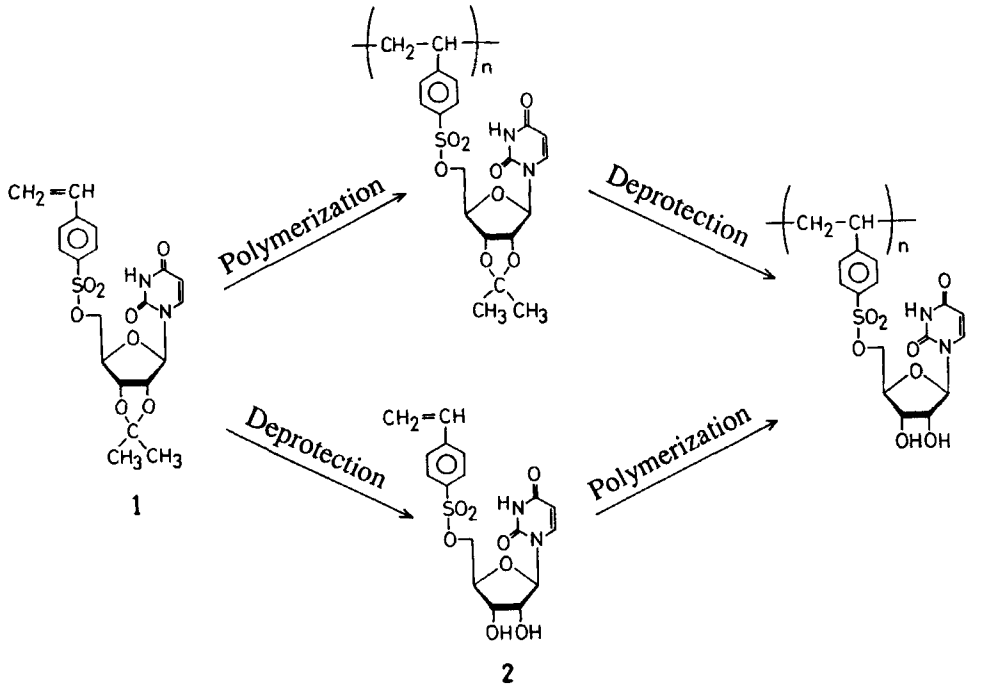

FIG. 3. Two synthetic routes to poly(uridine 5'-*p*-styrenesulfonate).

give the corresponding polymer in 38% yield. ^{1}H NMR spectrum of the polymer obtained is shown in FIG. 1. Peaks of the polymer main chain protons overlapped with the absorptions of the methyl protons of isopropylidene group.

The isopropylidene group of poly(**1**) was removed under acidic hydrolysis conditions, *i. e.*, the polymer in trifluoroacetic acid / water (5:1 v/v) and dimethyl sulfoxide (DMSO). Removal of the isopropylidene groups was confirmed by ^{1}H NMR spectroscopy. FIG. 2A shows that the methyl group peaks of the isopropylidene group (1.25 and 1.43 ppm) to be absent, indicating that the isopropylidene group was successfully cleaved to give poly(uridine 5'-*p*-styrenesulfonate). The number average molecular weight of the polymer was 2.0 x 10^5.

The poly(uridine 5'-*p*-styrenesulfonate) was synthesized also by the polymerization of deisopropylidenated monomer, *i. e.*, uridine 5'-*p*-styrenesulfonate (**2**), as shown in FIG. 3. Compound **2** was polymerized in DMSO with AIBN as an initiator at 70 °C under high vacuum for 2 days to give a polymer (25% yield) which had a number average molecular weight of 3.0 x 10^5. A detailed comparison of the ^{1}H NMR spectrum of poly(**2**) (FIG. 2B) with that of deprotected poly(**1**) (FIG. 2A) indicated that

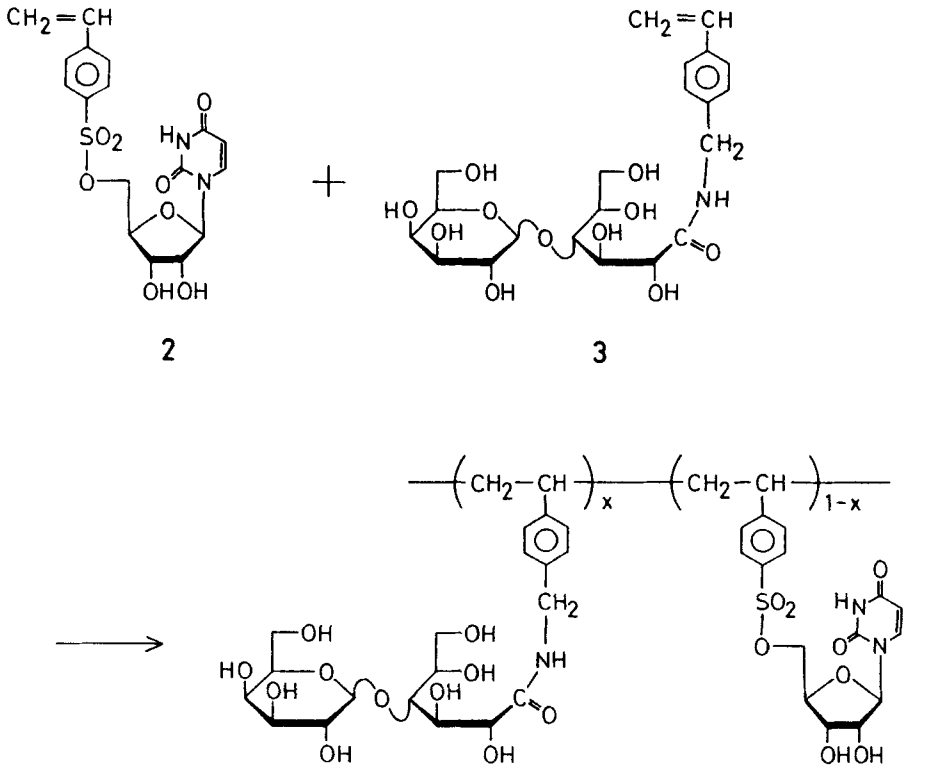

FIG. 4. Copolymerization of uridine-containing monomer with galactose-containing monomer.

the deprotected poly(**1**) had more irregular structure than poly(**2**), probably due to a side reaction of poly(**1**) with trifluoroacetic acid. Therefore, it may be said that the polymerization of deprotected monomer (**2**) gave the polymer having more regular structure.

Synthesis of Poly[(uridine 5'-*p*-styrenesulfonate)-*co*-{(*N*-*p*-vinylbenzyl-4-*O*-(β-D-galactopyranosyl)-D-gluconamide}]. Many kinds of glycosyltransferases located in Golgi complex and cell surface recognize UDP-sugars as glycosyl donors. In order to synthesize a polymer which can be recognized by glycosyltransferase, copolymerization of uridine-containing monomer (**2**) with galactose-containing monomer, *i. e.*, *N*-*p*-vinylbenzyl-4-*O*-(β-D-galactopyranosyl)-D-gluconamide (**3**) was carried out in DMF and in water as shown in TABLE 1. The mole fraction of each monomer in the feed was 0.5.

The ^{1}H NMR spectrum of the copolymer obtained in water is shown in FIG. 5. The absorptions assigned to both monomeric units (**2** and **3**) appeared in the spectrum of

TABLE 1. Copolymerization of **2** with **3**[a]

No.	Mole fraction of **2** in feed	Solvent	Yield (%)	Mole fraction of **2** in copolymer[b]	Mn[c] (x10^4)
1	0.5	DMF	9.2	0.39	3.8
2	0.5	water	15.6	0.38	3.8

a. Total monomer, 109 mg; solvent, 3 mL; temp, 70 °C; time, 24 h.
b. Determined by ^{1}H NMR spectroscopy.
c. Approximated by GPC.

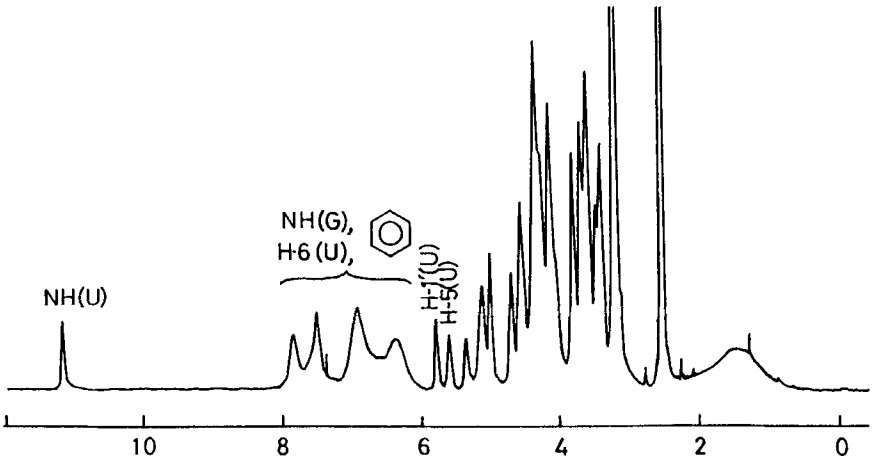

FIG. 5. ^{1}H NMR spectrum of copoly(**2**-**3**). (U): uridine residue; (G): galactosyl gluconamide residue.

the copolymerization product. The absorption at 5.8 ppm is due to the H-1' proton of ribose in the uridine residue, and the absorptions at 6 - 8 ppm are due to the aromatic protons of both styrene groups and H-6 proton of uridine residue and NH proton of lactonamide residue. Accordingly, the copolymer composition was calculated by using the relative intensities of the absorptions.

In order to synthesize the poly[(uridine 5'-*p*-styrenesulfonate)-co-{(*N*-*p*-vinylbenzyl-4-*O*-(β-D-galactopyranosyl)-D-gluconamide}], deprotection of the copolymer which had been prepared by the copolymerization of **1** with **3** was also attempted, but the removal of galactosyl gluconyl residue occurred.

Study of the interaction between the new synthetic copolymers and biomolecules such as galactosyltransferase is now in progress.

EXPERIMENTAL

2',3'-*O*-Isopropylideneuridine 5'-*p*-Styrenesulfonate (1). 2',3'-*O*-Isopropylideneuridine (SIGMA Chemical Co., U.S.A.) (100 mg, 0.35 mmol) and *p*-styrenesulfonyl chloride (230 mg, 1.13 mmol) which had been synthesized by the reaction of sodium *p*-styrenesulfonate and thionyl chloride were dissolved in pyridine (2 mL) at 0 °C under high vacuum and maintained at 0 °C. After 2 h, the solution was poured into cold water saturated with sodium hydrogen carbonate. The product was extracted with chloroform, and the chloroform solution was washed with cold water, dried on sodium sulfate, and concentrated to dryness. The syrupy product was chromatographed on silica gel, with chloroform-acetone (5:1 v/v) as eluent, and was purified by precipitation using the acetone-hexane system to afford powdery 2',3'-*O*-isopropylideneuridine 5'-*p*-styrenesulfonate (**1**) (63.5 mg, 40.3%): ^{1}H NMR (DMSO-d_6) δ 11.40 (s, 1H, NH), 7.83 (d, 2H, J = 7.8 Hz, aromatic), 7.70 (d, 2 H, aromatic), 7.62 (d, 1H, $J_{5,6}$ = 7.9 Hz, H-6), 6.82 (dd, 1H, J_{AX} = 17.6 Hz, J_{BX} = 11.0 Hz, CH_2=C<u>H</u>Ph), 6.04 (d, 1H, C<u>H$_A$</u>H$_B$=CHPh), 5.71 (s, 1H, H-1'), 5.57 (d, 1H, H-5), 5.49 (d, 1H, CH$_A$<u>H$_B$</u>=CHPh), 5.02 (d, 1H, $J_{2'3'}$=6.3 Hz, H-2'), 4.71 (m, 1H, H-3'), 4.2-4.4 (2H, overlapped, H-5'a, H5'b), 4.15 (1H, m, H-4'), 1.43 (s, 3H, isopropylidene), 1.25 (s, 3 H, isopropylidene).

Anal. Calcd for $C_{20}H_{22}O_8N_2S$ (450.5): C, 53.33; H, 4.92; N, 6.22; S, 7.12. Found: C, 53.48; H, 4.87; N, 6.25; S, 7.46.

Uridine 5'-*p*-Styrenesulfonate (2). The isopropylidene group of **1** (0.9 g) was removed by hydrolyzing **1** in 8 mL of trifluoroacetic acid-water (7:1 v/v). After the solution was magnetically stirred at room temperature for 30 min, it was concentrated at 40 °C and the product was dried *in vacuo*. The product was dissolved in water and the solution was filtered to remove the water-insoluble byproduct, washed with chloroform, and finally freeze-dried to give uridine 5'-*p*-styrenesulfonate (**2**) (160 mg, 20.1%): ^{1}H NMR (DMSO-d_6) δ 11.18 (s, 1H, NH), 7.87 (d, 2H, J = 8.4 Hz, aromatic), 7.74 (d, 2 H, aromatic), 7.44 (d, 1H, $J_{5,6}$ = 7.9 Hz, H-6), 6.85 (dd, 1H, J_{AX} = 17.7 Hz, J_{BX} = 11.1 Hz, CH_2=C<u>H</u>Ph), 6.03 (d, 1H, C<u>H$_A$</u>H$_B$=CHPh), 5.71 (d, 1H, $J_{1,2}$ = 4.9 Hz, H-1'), 5.57 (d, 1H, H-5), 5.51 (d, 1H, CH$_A$<u>H$_B$</u>=CHPh), 3.9-4.4 (5H, overlapped, H-2', H-3', H-4', H-5'a, H5'b).

Anal. Calcd for $C_{17}H_{18}O_8N_2S$ (410.4): C, 49.75; H, 4.42; N, 6.83; S, 7.81. Found: C, 48.27; H, 4.56; N, 6.15; S, 7.38.

***N*-*p*-vinylbenzyl-4-*O*-(β-D-galactopyranosyl)-D-gluconamide (3).** *N*-*p*-Vinylbenzyl-4-*O*-(β-D-galactopyranosyl)-D-gluconamide (**3**) was synthesized by the reaction of *p*-vinylbenzylamine with 4-*O*-(β-D-galactopyranosyl)-D-glucono-1,5-lactone which was prepared by the oxidation of D-lactose, according to the literature.[1]

Polymerization. Homopolymerizations of **1** and **2** were carried out in DMF and DMSO, respectively, with AIBN as initiator at 70°C under high vacuum. Poly(**1**) was purified by reprecipitation using DMF-THF system and dried *in vacuo*. Poly (**2**) was purified by gel filteration with Sephadex G-50, and then finally freeze-dried from water. Copolymerizations of **1** with **3** and **2** with **3** were carried out in the same manner as homopolymerizations.

Deprotection of Polymers. Isopropylidene groups of 20 mg of poly(**1**) were removed by hydrolyzing the polymer in 86 μL of 5:1 (v/v) trifluoroacetic acid/water and 1 mL of DMSO. After the solution was magnetically stirred at room temperature for 10 h, it was concentrated and diluted with water. The deprotected polymer was purified by reprecipitation using water-methanol system and gel filteration with Sephadex G-50, and then freeze-dried from water. Deprotection of the copolymer of **1** with **3** was attempted in the same manner as above.

General Procedures. NMR spectra were recorded on a JEOL EX-270 spectrometer in DMSO-d_6. The number-average molecular weights of water-soluble polymers were determined by aqueous phase GPC (columns: Asahipak GS-510 (Asahi Chemical Industry)) using standard pullulans as reference.

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