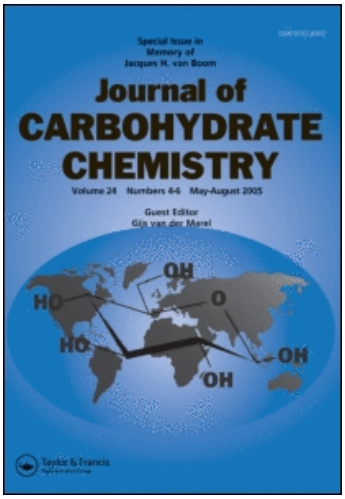


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# *O*-(Hydroxypropyl)Sucrose

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# *O*-(HYDROXYPROPYL)SUCROSE

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## ABSTRACT

A laboratory procedure for making *O*-(hydroxypropyl)sucrose preparations containing predominantly, in decreasing order, derivatives containing one, two, and three hydroxypropyl ether groups has been developed. The product was noncrystalline. A sample which contained primarily mono- and di-hydroxypropylated sucrose molecules gave a conservative maximum of 3% cleavage when treated with invertase (ß-fructofuranosidase) and sucrase (sucrose α-D-glucohydrolase) during an 18 h digestion, indicating possible application as a reduced-calorie bulking agent. Incubation with a culture of fecal bacteria indicated no metabolism of *O*-(hydroxypropyl)sucrose by, and no toxicity to, colonic microorganisms. One chromatographically pure fraction was determined to be 6-*O*-(hydroxypropyl)sucrose.

## INTRODUCTION

A food bulking agent with little or no caloric value should have the following characteristics: good water solubility, but forming low-viscosity solutions; tasteless or slightly sweet (not sweeter than sucrose nor bitter); stable to conditions of food processing and preparation; nondigestible and nonabsorbable by humans; nonutilizable by colonic microorganisms, i.e., will not produce flatulence or diarrhea. A search for a reduced-calorie bulking agent began with the assumption that slight derivatization of sucrose will prohibit its enzyme-catalyzed hydrolysis, the initial reaction in any biological utilization, while retaining

its other desirable properties. The criteria established for this preparation were as follows: any reaction must be done in water or without solvent because water is the only effective, economical, and food-safe solvent for sucrose; only bases can be used as catalysts because of the acid lability of sucrose; the derivative must be physiologically safe. Derivatization by addition of the hydroxylpropyl group was selected because hydroxypropylstarch and hydroxypropylcellulose are approved food additives. (For food use, hydroxypropylstarch may contain up to 25% and hydroxypropylcellulose may contain up to 80.5% hydroxypropyl groups. Propylene glycol esters of alginic acid and various fatty acids and propylene glycol itself may also be used.[1]) The objectives of this work were, therefore, to react sucrose with propylene oxide to make products with low degrees of substitution (DS) and to characterize the products chemically and physiologically.

An additional objective of the project was to determine if the known ability of sucrose to complex metal ions could be used to provide regioselectivity to the hydroxypropylation reaction. The presence, in neutral solution, of 2sucrose•3NaI•$3H_2O$ and sucrose•NaI•$2H_2O$ has been demonstrated and their effect on the solubility of each component has been determined.[2] Earlier, crystals of sucrose•NaI•$H_2O$ were actually isolated.[3] In aqueous solutions of low to moderate concentration of salt, NaI, KI, NaBr, KBr, NaCl, and KCl form preponderantly 1:1 sucrose-alkali metal salt complexes.[4,5] With alkaline earth metal salts, both 1:1 and 2:1 sucrose-salt adducts were indicated.[5] Sucrose is soluble in methanol containing calcium chloride.[6]

Sucrose will complex with and solubilize calcium hydroxide; for example, at 25 °C, lime (determined as CaO) is 77.5 times more soluble in a 34% sucrose solution (the concentration used here) than it is in water.[7] It has also been shown that, when an aqueous solution of sucrose and calcium oxide is added to an aqueous solution of sodium aluminate, a water-soluble complex containing sucrose, aluminum, and calcium is formed.[8] Phase studies of various ternary systems have revealed the presence (in aqueous systems) of sucrose•BaO, sucrose•3BaO, sucrose•SrO, sucrose•2SrO,[9] and sucrose $Na_2CO_3$.[10]

Dow Chemical Co. makes polyether polyols by extensive reaction of sucrose with propylene and ethylene oxides and markets them as intermediates for the preparation of urethane elastomers, coatings, and flexible and rigid foams under the trade name Voranol. Similar products have been made by reacting sucrose with propylene oxide under heat and pressure in the presence of a base and a very small amount of water.[11] A similar product was made from the lactose in whey permeate,[11] but in that case the disaccharide was cleaved so that the product contained monosaccharide ethers.[12] Polyether polyols have also been made by reaction of lactitol with propylene oxide.[13] However, no *O*-(hydroxypropyl)sucrose preparations of low DS have been previously reported.

After completion of this work, a patent appeared that describes a similar product made from starch.[14] The patent covers hydrolyzates of *O*-(hydroxypropyl)starch containing more than ca. 15% by weight of DP 2-6 oligomers which are useful as reduced-calorie replacements for sucrose and starch hydrolyzate products.

## RESULTS AND DISCUSSION

A standard laboratory procedure for preparation of *O*-(hydroxypropyl)sucrose was developed. When sucrose was reacted with propylene oxide (PO) in the presence of different amounts (0.05 to 0.5 M) of NaOH, $Ca(OH)_2$, or $Ba(OH)_2$, similar products were obtained, suggesting that pH is more important than the nature of the cation and that the cation does not provide specificity. Calcium hydroxide at pH 12.55 was generally used thereafter as the base because of greater ease in removing it at the end of the reaction. The reaction was more efficient at high temperatures, but at higher temperatures a greater proportion of more highly substituted hydroxypropylsucrose products (as indicated by TLC) was formed. Therefore, the reaction was done at 20-25 °C. At that temperature, ca. 24 h were required to consume all the PO. PO:sucrose ratios of 1:1 to 5:1 were used. A ratio of 2:1 gave the least unreacted sucrose and the least highly substituted sucrose, i.e., was optimum for the formation of mono- and disubstituted sucrose (as indicated by TLC). The basic method described under Materials and Methods (referred to as the standard method) was designed to minimize formation of poly(propylene oxide) chains on the sucrose molecules. It, however, results in a reaction mixture that contains unreacted sucrose molecules.

On a small scale, unreacted sucrose was removed from the product by applying a neutral, concentrated, aqueous solution to a column of Amberlite XAD-16, XAD-4, or XAD-2 resin. Amberlite XAD-16 resin was preferred for separation of unreacted sucrose from the product. When the column was washed with deionized water, unreacted sucrose appeared first, followed by mono-*O*-(hydroxypropyl)sucrose. The remaining hydroxypropylsucrose was eluted with 50% ethanol. On a larger scale, unreacted sucrose was removed from the product using yeast.

Sucrose and hydroxypropylsucrose were incubated with invertase and porcine intestine acetone powder as a source of sucrase (sucrose α-D-glucohydrolase). Yeast invertase gave 100% conversion of sucrose into glucose and fructose. The porcine intestine powder gave less than complete theoretical yields of D-glucose and D-fructose from sucrose. Little hydroxypropylsucrose was cleaved by either enzyme in 6 or 18 h (Table 1). During the incubation period, hydroxypropylsucrose gave a conservative maximum of 3% cleavage of the

TABLE 1. Hydrolysis of Hydroxypropylsucrose via Incubation with Yeast Invertase and Porcine Intestine Sucrase at 35°C for 18 h With Shaking

| Substrate[a] | Hydrolysis products (mg/mL) | | | |
|---|---|---|---|---|
| | Invertase | | Intestine powder | |
| | Glc | Fru | Glc | Fru |
| Sucrose (control) | 18.6[b] | 18.6[b] | 10.3[b] | 13.7[b] |
| HP-sucrose[c] | 0.15 | 0.38 | 0 | 0 |
| HP-sucrose[d] | 0.16 | 0.39 | 0 | 0 |

a. Concentrations = 0.1 M
b. Theoretical = 18.0
c. From a reaction with a 4:1 ratio of PO:sucrose. Sucrose removed via incubation with yeast.
d. From a reaction with a 10:1 ratio of PO:sucrose. Sucrose removed via incubation with yeast.



theoretical value when treated with invertase and sucrase (Table 1). The same sort of data was obtained when sucrose was removed with an Amberlite XAD-4 column.

Metabolism of substances by colonic microorganisms occurs via fermentative pathways not requiring oxygen and results in the production of short-chain acids. A common way to determine if a substance can be utilized by these organisms is to monitor acid production (drop in pH) during an anaerobic incubation with them. Hydroxypropylsucrose showed neither any detectable metabolism by fecal bacteria nor any toxic effect to them under anaerobic conditions during a 72 h monitoring period (Fig. 1). D-Glucose was used as a positive control and produced a rapid drop in medium pH (from pH 6.8 to pH 4.2 in 48 h). Hydroxypropylsucrose samples, which were not completely free of unreacted sucrose, showed little or no pH change (a maximum drop to pH 6.6). Neither did HPLC chromatograms change with incubation, except for removal of traces of unreacted sucrose.

Unreacted sucrose was separated from the reaction product by eluting it with water from an Amberlite XAD-16 column, on which it was slightly retained. Following sucrose, the column eluant contained seven monosubstituted components (by HPLC and FAB-MS) which could be separated into four mono-*O*-(hydroxypropyl)sucrose fractions containing 2, 1, 2 and 2 components as determined by HPLC. The main fraction was the only one characterized and identified as 6-*O*-(hydroxypropyl)sucrose by $^{13}C$ NMR, the signal at $\delta$ 62.11 for C-6 shifting downfield after hydroxypropylation (Table 2). Since a clean spectrum was obtained from a fraction containing a pair of compounds, it is assumed that the compounds are R and S isomers (of the hydroxypropyl group). It is likely that the other fractions containing two components, likewise contain pairs of R and S isomers.

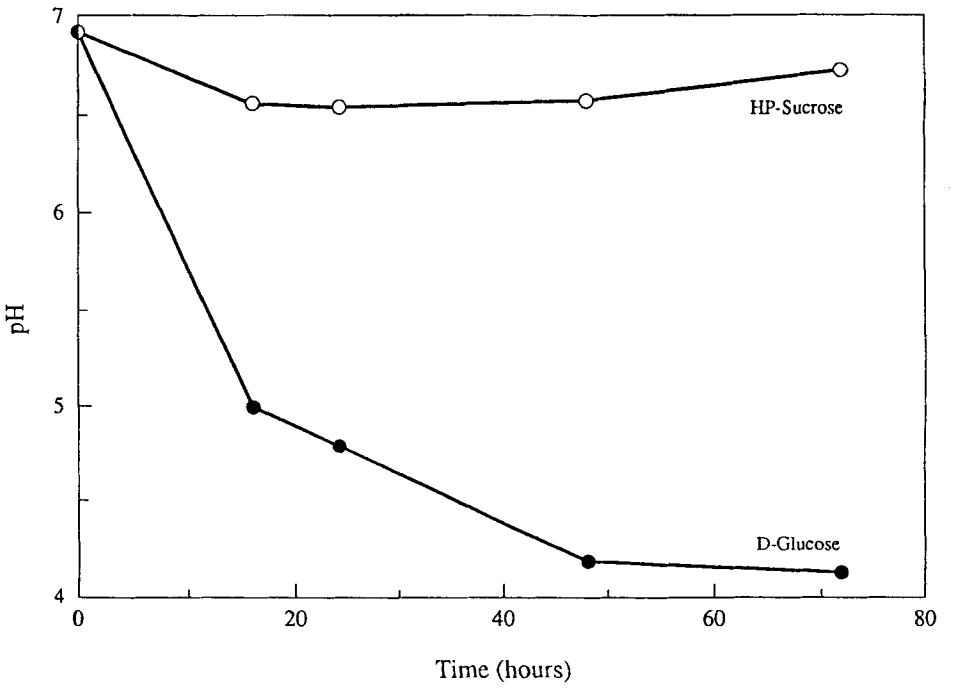


Fig. 1. A typical caloric density assay result from a hydroxypropylsucrose preparation.



NMR (both $^{1}H$ and $^{13}C$) spectral analysis has previously been used to locate methyl ether groups of *O*-methylsucroses;[15,16] to determine the positions of acylation of sucrose esters;[17-19] to determine the molar substitution, degree of substitution, and location of substituent ether groups of *O*-(hydroxypropyl)-, *O*-(hydroxyethyl)-, and *O*-(carboxymethyl)celluloses;[20-25] to locate the carboxymethyl groups of *O*-carboxymethyl ethers of D-glucose;[26] and to locate and quantitate the acetyl groups of a polysaccharide.[27] Permethylation analysis has been used to locate the hydroxypropyl groups of *O*-(hydroxypropyl)guaran[28] and *O*-(hydroxypropyl)cyclomaltoheptaose.[29]

Gradient elution of hydroxypropylsucrose from an Amberlite XAD-16 column using 0-20% ethanol in water separated it into three components (by TLC). FAB-MS revealed the three fractions to be mono-, di-, and tri-*O*-(hydroxypropyl)sucroses. Some fragmentation occurred and peaks for substituted glucosyl or fructosyl units could be assigned based upon knowledge that fragmentation yields glucose and the fructosyl cation. There is evidence that acid-catalyzed and pyrolytic cleavage of the glycosidic bond of sucrose produces a fructofuranosyl cation and a glucosyloxy anion.[30] Based on the assumption that the same cleavage occurs during FAB mass spectrometry, peaks were obtained for the following

TABLE 2. $^{13}C$ NMR Spectral Data for Mono-*O*-(hydroxypropyl)sucrose Fraction I from an Amberlite XAD-16 Column

| Carbon atom[a] | Chemical shift (ppm) | |
|---|---|---|
| | Mono-*O*-(hydroxypropyl)-sucrose | Mono-*O*-(hydroxypropyl)-sucrose + Sucrose |
| G1 | 92.78 | 92.92 |
| G2 | 71.88 | 71.87 |
| G3 | 73.58 | 73.57 |
| G4 | 70.05 | 70.00 |
| G5 | 73.09 | 73.08 |
| G6 | 62.93 | 62.95, 62.11 |
| F1 | 63.21 | 63.16 |
| F2 | 104.59 | 104.53 |
| F3 | 77.15 | 77.17 |
| F4 | 74.28 | 74.27 |
| F5 | 82.07 | 82.10 |
| F6 | 63.38 | 63.36 |
| C1 | 60.89 | 60.91 |
| C2 | 84.96 | 84.94 |
| C3 | 18.92 | 18.92 |

[a]G = α-D-glucopyranosyl unit, F = ß-D-fructofuranosyl unit, C = hydroxypropyl unit.



fragments containing the specified number of hydroxypropyl groups: m/z 221 ($M+H^+$) Fru-1HP; 279 ($M+H^+$) Fru-2HP; 337 ($M+H^+$) Fru-3HP; 203 ($M+Na^+$) Glc-0HP; 261 ($M+Na^+$) Glc-1HP; 319 ($M+Na^+$) Glc-2HP. Other peaks representing loss of one water molecule from each of the above, including 163 ($M+H^+$-HOH) Fru-0HP, were also observed. In this way, entire preparations were indicated to be mixtures containing molecules with 0, 1, and 2 hydroxypropyl ether groups on the D-glucosyl unit and 0, 1, 2, and 3 hydroxypropyl ether groups on the D-fructosyl unit; mass spectrometry did not provide a determination of the ratio of D-glucosyl units with two single hydroxypropyl ether groups to those with one chained ether group, for example. Therefore, a mixture of *O*-allylsucroses was prepared in a similar way and converted into *O*-(hydroxypropyl)sucrose via oxymercuration[31] followed by *in situ* treatment with sodium borohydride.[32] Similar chromatographic profiles and mass spectra indicated that little chaining had occurred in the propylene oxide reaction.

It is concluded that four mono-*O*-(hydroxypropyl)sucrose derivatives were formed, that each represented a different site of substitution (one of which was determined to be O-6 of the D-glucosyl unit), and that each sucrose derivative was composed of an RS pair of hydroxypropyl ether groups.

## EXPERIMENTAL

**Materials.** The following materials were obtained from the sources indicated: Amberlite XAD-7, XAD-4, and XAD-2 resins (Aldrich Chemical Co.), Amberlite XAD-16 resin (Supelco, Inc.), Amberlite IR-120, medium-porosity, cation-exchange resin (Aldrich Chemical Co.), Amberlite IRA-93, macroporous-type, weakly basic anion-exchange resin (Sigma Chemical Co.), propylene oxide (Baker Analyzed), yeast invertase (ß-fructofuranosidase) (Boehringer Mannheim), porcine intestine acetone powder (Sigma Chemical Co.), yeast extract, malt extract, and Bacto-Peptone (Difco Laboratories).



**Preparation of *O*-(Hydroxypropyl)sucrose.** To a round-bottom flask, $Ca(OH)_2$ (0.74 g, 0.01 mol), sucrose (6.84 g, 0.02 mol), and water (20 mL) were added. With stirring at room temperature, the calcium hydroxide dissolved to give a solution that was 0.5 M in $Ca(OH)_2$ and 1.0 M in sucrose. Propylene oxide (2.7 mL, 0.04 mol) was added, and stirring at room temperature (ca. 25 °C) was continued for 24 h. Following filtration, the filtrate was passed through a column of Amberlite IR-120($H^+$) cation-exchange resin at 4 °C. The void volume was discarded, and the portion of the eluate that contained product (pH 4) was collected. That fraction was passed immediately through a column of Amberlite IR-93($OH^-$) anion-exchange resin at room temperature to adjust the pH to ca. 8. The solution was then concentrated under reduced pressure at 50 °C.

Alternatively, the reaction mixture was neutralized and most of the $Ca^{2+}$ removed by treating the solution with $CO_2$ and removing the precipitate of $CaCO_3$ by filtration.

Sucrose was also reacted with propylene oxide in the presence of different amounts of either NaOH, $Ca(OH)_2$, or $Ba(OH)_2$, at different temperatures, and with propylene oxide: sucrose ratios varying from 1:1 to 10:1.

**Separation of Unreacted Sucrose from *O*-(Hydroxypropyl)sucrose via Column Chromatography.** Amberlite XAD-2, XAD-4, and XAD-16 resins were used to remove unreacted sucrose. Columns were washed first with water to elute sucrose, then with 50% ethanol to elute hydroxypropylsucrose. The 50% ethanol eluate was concentrated under reduced pressure at 50 °C to a colorless and odorless syrup free of sucrose that could be converted to a grindable glass by drying *in vacuo*.

**Separation of Unreacted Sucrose from *O*-(Hydroxypropyl)sucrose via Incubation with Yeast.** Bakers' yeast was grown in 100 mL of sterilized medium containing 0.3 g of yeast extract, 0.3 g of malt extract, and 0.5 g of Bacto-Peptone at 37 °C for 48 h, the medium being changed after 24 h. Hydroxypropylsucrose syrup (ca. 6 g dry weight) was dissolved in 100 mL of water; 0.3 g of yeast extract, 0.3 g of malt extract, and 0.5 g of Bacto-Peptone were added, and the medium was sterilized. The yeast was collected by centrifugation and added to the medium, which was then shaken gently at 37 °C for 48 h. The yeast was removed by filtration or centrifugation. The solution contained no sucrose (TLC), glucose or fructose (test kit containing HK/G6PDH and PGI, Boehringer Mannheim). The hydroxypropylsucrose solution was passed first through a column of Amberlite IR-120($H^+$), then a column of Amberlite IRA-93($OH^-$) to remove the added materials. The final eluate (pH ca. 8) was concentrated under reduced pressure at 50 °C to a syrup identical to that obtained by column chromatography.

**Digestion of *O*-(Hydroxypropyl)sucrose by Yeast Invertase and Porcine Intestine Sucrase.** Sucrose (control) and hydroxypropylsucrose (substrate) were incubated at concentrations of 0.1 M with invertase in 0.32 M citrate buffer solution, pH 4.6, and with porcine intestine powder in 0.1 M maleate buffer, pH 6.8, in an incubator-shaker at 35 °C for 18 h. The products of hydrolysis (glucose and fructose) were determined with a glucose/fructose test kit (Boehringer Mannheim).

**Fecal Fermentation Assay of *O*-(Hydroxypropyl)sucrose.** A hydroxypropylsucrose preparation was examined using a standard fecal fermentation assay protocol.

**Separation of Components of *O*-(Hydroxypropyl)sucrose Preparations.** The components of hydroxypropylsucrose preparations were resolved using Amberlite XAD-16 resin (100 g) in a glass column (l = 55 cm, d = 2 cm). The column was first washed with 95% ethanol and water. Hydroxypropylsucrose (0.9 g) was dissolved in 2 mL of $H_2O$, and ca. 5 g of Amberlite XAD-16 resin was added to the solution. The mixture was shaken and filtered. The resin, with the hydroxypropylsucrose bound to it, was loaded onto the column, which was then washed with a gradient of from 0% to 20% ethanol in water. Eluent (5-mL fractions) was collected in each tube with a flow rate of 1 drop/5 sec. Tubes were checked by TLC. Three fractions were formed by pooling; each was analyzed by fast atom bombardment mass spectrometry (FAB-MS).

***O*-(Hydroxypropyl)sucrose from *O*-Allylsucrose.** *O*-Allylsucrose was prepared under the same general conditions used for the preparation of *O*-(hydroxypropyl)sucrose. Allyl bromide (0.4 mol) was added dropwise to 100 mL of a 1.0 M solution of sucrose in 0.5 M $Ca(OH)_2$. The reaction was allowed to proceed for 25-43 h at room temperature. Thin-layer

chromatography suggested that the product was a mixture of sucrose molecules with 0, 1, 2, 3, and 4 allyl ether groups. The reaction mixture was worked up in the same way as was the hydroxypropylsucrose reaction mixture, including removal of unreacted sucrose using an Amberlite XAD-4 resin column. Pure mono-*O*-allylsucrose (FAB-MS) was eluted from the column with 5% ethanol.

Both the mono-*O*-allylsucrose fraction and the total product mixture were converted into *O*-(hydroxypropyl)sucrose mixtures via oxymercuration[12] followed by reduction with sodium borohydride.[13]

**HPLC.** HPLC analysis was conducted with a Dionex Bio LC Carbohydrate system equipped with a column (4.6 X 250 mm) of Dionex Carbopac AS-6 pellicular anion-exchange resin, an AG-6 guard column, and the pulsed amperometric detector (PAD). The Dionex eluant degas module was employed to sparge and pressurize the eluants with helium. Eluant 1 was 500 mM NaOH; eluant 2 was water, and eluant 3 was 50 mM NaOH. Water used was demineralized and purified by filtration via a Millipore installation (Millipore, Bedford, MA) consisting of one prefilter cartridge, one super cartridge, and two ion-exchange cartridges. The sample solution was filtered through an Aerodisc LC13 PVDF syringe filter (13 mm diam and 0.2 $\mu$m pore size); 15 $\mu$L was injected. Sample injection was done via a Dionex microinjection valve equipped with a 25-$\mu$L sample loop operated by a controlled helium source of 100-120 psi. Analysis of hydroxypropylsucrose was done at an isocratic NaOH concentration of ca. 15 mM (i.e., 30% of eluant 3 and 70% of eluant 2) for 1 min; followed by a gradient of from 100% of eluant 2 to 100% of eluant 3 for 30 min at a flow rate of 1 mL/min at ambient temperature. A 5-min column wash with 500 mM NaOH followed by a 10-min equilibration with 15 mM NaOH (30% of eluant 3 and 70% of eluant 2) at a flow rate of 2 mL/min at ambient temperature was required to yield reproducible retention times.

Separated monosaccharides were detected by PAD using a gold working electrode. In order to minimize baseline distortion due to change in pH of the eluant, 400 mM NaOH was added to the postcolumn effluent via a mixing tee at a flow rate of 1 mL/min using the Dionex Autoion reagent pump. The following pulse potentials and durations were used: E1 = 0.05V ($t_1$ = 360 ms); E2 = 0.80V ($t_2$ = 120 ms); E3 = -0.60V ($t_3$ = 420 ms).

**FAB-MS.** A double-focusing Kratos MS 50S spectrometer equipped with the standard FAB source and a DS 90 data system was used to obtain mass spectra. The Kratos FAB gun was operated with a 7 Kv xenon beam. The instrument was scanned from m/z 1000 to 40 with a scan rate of 10 s/decade. Spectra were obtained using a mixture of dithiothreitol (DTT) and dithioerythritol (DTE) (1:1 w/w) as the matrix (DTT/DTE).

**NMR.** All spectra were obtained on a GE QE 300 MHz spectrometer operating at a spectral frequency of 75.607311 Hz for $^{13}C$ with the probe at ambient temperature.

Deuterium oxide was used to dissolve the sample. Dioxane was used as an internal standard for chemical shift reference.

**TLC.** TLC was conducted on silica gel 60 pre-coated aluminum sheets (E. Merck). The solvent system used for chromatography was 4:5:1 (v/v) 1-butanol-acetone-water. Components on TLC plates were detected by spraying with 15% (v/v) sulfuric acid in 50% (v/v) aqueous ethanol and heating for several minutes at ca. 150 °C.

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