Synthesis and reactions of uronic acid derivatives. XII.* Synthesis of methyl[methyl 2-*O*-(2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl)-3,4-di-*O*-methyl-α-D-galactopyranosid]uronate, a fully methylated β-D-1→2-linked pseudoaldobiouronic acid

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A good yield of crystalline pseudoaldobiouronic acid derivative *III* was obtained by allowing to react 2,3,4,6-tetra-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranose with methyl(methyl 3,4-*O*-isopropylidene-α-D-galactopyranosid)uronate under the conditions of modified Königs—Knorr synthesis of glycosides. The alcoholic hydroxyl groups in *III* were regenerated by selective deisopropylidenation and subsequent deacetylation giving the crystalline methyl ester methyl glycoside *V*. Methylation of *V* with methyl iodide and silver oxide in *N,N*-dimethylformamide, which gave the crystalline title derivative *VII* as the main product, was accompanied by a β-elimination reaction forming the olefinic disaccharide *X*. Olefin *X* was also synthesized and obtained crystalline by directed β-elimination reaction of *III* followed by methylation of the deacetylated, crystalline olefin *IX*. The structures of *VII*, *X* and their synthetic precursors were confirmed by mass spectrometry.

Конденсацией 2,3,4,6-тетра-*O*-ацетил-1-бромо-1-дезокси-α-D-глюкопиранозы с метил(метил-3,4-*O*-изопропилиден-α-D-галактопиранозид)уронатом при условиях модифицированной реакции Кенигса—Кнорра получилось в хорошем выходе производное псевдоальдобиуроновой кислоты *III*. Устранение блокирующих групп в *III* было сделано селективной деизопропилиденацией и последовательным деацетилированием дал кристаллический метил эфир метил гликозид *V*. Его метилирование, которым получено кристаллическое титулярное вещество *VII*, иодистым метилом и окисью серебра в *N,N*-диметилформамиде сопровождалось побочной реакцией β-элиминирования с возникновением олефинового дисахарида *X*. Олефин *X* был тоже приготовлен и получен в кристаллическом виде реакцией β-элиминирования выполненой на *III* и метилированием образовавшегося кристаллического олефина *IX*. Структуры *VII*, *X* как и большинства промежуточных продуктов их синтеза были подтверждены интерпретацией их масс-спектров.

Studies on the formation of olefinic products during methylation of uronic acid derivatives and mass spectral investigations on the differences between the

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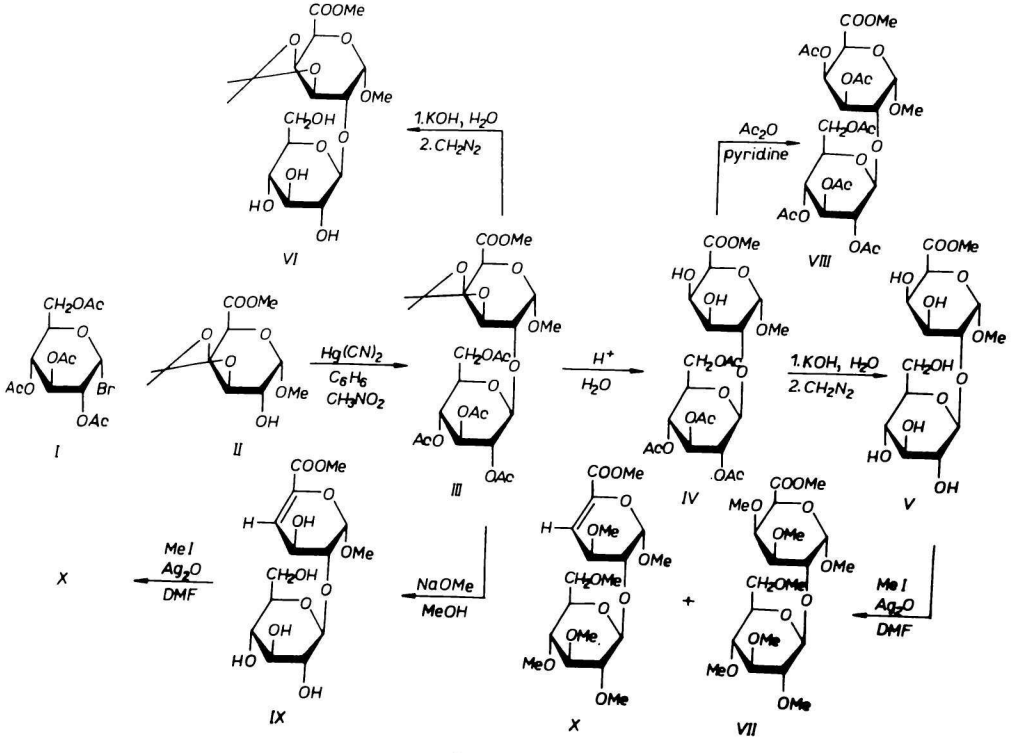
fragmentation of methylated aldobiouronic and pseudoaldobiouronic acids required a series of acidic oligosaccharides. The present paper describes the synthesis of the first member in the series, carried out according to Scheme 1.



Scheme 1

Experimental

Melting points were determined on a Kofler hot-stage. Optical rotations were measured using a Perkin—Elmer automatic polarimeter, Model 141. Mass spectra were obtained with a JMS 100 D instrument using direct sample introduction into the ion source. The temperature in the site of evaporation varied according to the volatility of the substances from 150 to 180°C and the electron energy, at an emission of 300 μA, was 70 eV.

Thin-layer chromatography (t.l.c.) on Silica Gel G coated glass slides and chromatography on columns of dry-packed silica gel (Merck, A. G., Darmstadt, product No. 9385) were done with: *A.* benzene—acetone 5:1, *B.* benzene—acetone 8:1, *C.* chloroform—acetone 5:1, *D.* chloroform—acetone 10:1, *E.* chloroform—methanol 4:1, and *F.* chloroform—methanol 10:1. The solvent ratios are based on volumes. Prior to packing the silica gel was equilibrated with 40% (v/w) of the mobile phase. Detection was performed by *a*) spraying with 5% sulfuric acid in ethanol and heating until permanent char spots were visible, and *b*) spraying with 0.1% potassium permanganate in acetone which immediately revealed the olefinic components as yellow spots on violet background.

Silver oxide was freshly prepared as described in [1]. *N,N*-Dimethylformamide and methyl iodide were purified as recommended by *Perrin et al.* [2]. Other chemicals were commercial products of reagent grade purity used as supplied. Unless otherwise stated, concentration of the solutions was carried out at 2 kPa and 40°C.

Methyl[methyl 2-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-3,4-O-isopropylidene-α-D-galactopyranosid]uronate (III)

Compound *II* (2.5 g; 10 mmoles) was dissolved in benzene—nitromethane (1 : 1; 250 ml). Drierite (10 g) and mercuric cyanide (2.5 g; 10 mmoles) were added and 70 ml of the solvents was distilled off at atmospheric pressure and with exclusion of moisture. The mixture was cooled to room temperature and, after an addition of bromide *I* (4.1 g; 10 mmoles), the reaction mixture was stirred for 16 hrs. A fresh portion of *I* (1 g) was introduced, followed by another one (1 g) after 8 hrs the stirring being continued for a total of 24 hrs, at which time t.l.c. showed the absence of *I* (R_F 0.65, solvent *A*). The mixture was filtered, the filtrate containing besides a small amount of *II* (R_F 0.25) mainly the product *III* (R_F 0.38), was combined with the chloroform washings and concentrated. The solution of the residue in chloroform was washed with aqueous 1 M potassium bromide until the test for the presence of mercuric ions in the water phase was negative. The chloroform solution was washed with water, dried with anhydrous sodium sulfate, concentrated, and the crude product (8.2 g) was chromatographed on a column of silica gel (75 × 4 cm). Elution with solvent *B* gave 3.95 g (70%, based on *II*) of chromatographically pure *III* which, when crystallized from ether, melted at 150—152°C. Ref. [3] gives m.p. 151—152°C.

Methyl[methyl 2-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-D-galactopyranosid]uronate (IV)

Water (45 ml) was added with stirring to a solution of *III* (4.5 g) in glacial acetic acid (45 ml) and the reaction mixture was kept at 60°C for 2 1/2 hrs. T.l.c. (solvent *C*) showed then that only small amount of the starting material (R_F 0.8) was present and, since besides the main component *IV* (R_F 0.3) the mixture contained also slower moving decomposition products, the reaction was terminated by concentration and evaporation of the residue with several portions of water and methanol, to remove acetic acid. The obtained syrup was chromatographed on a column of silica gel (50 × 4.5 cm) with solvent *C* to give 3.5 g (83.4%) of chromatographically pure *IV* which crystallized spontaneously on standing overnight at room temperature. Crystallization from methyl acetate—ether (2 : 1) gave material which, when dried at 40°C/2 kPa. melted partially at 80—90°C, solidified on further heating and then melted sharply at 167—168°C. The observed double melting point and $[\alpha]_D^{23}$ +49.5° (*c* 1.06, methanol) has not changed on recrystallization from the same solvent. When dried at 100°C/2 kPa for 1 hr *IV* melted at 167—169°C. Recrystallization from ethanol, after seeding with the higher melting material gave *IV* melting sharply at 167—168°C and having $[\alpha]_D^{23}$ +49° (*c* 1.03, methanol).

For $C_{22}H_{32}O_{16}$ (552.58) calculated: 47.82% C, 5.84% H; found: 47.74% C, 5.90% H.

Methyl[methyl 2-O-(β-D-glucopyranosyl)-α-D-galactopyranosid]uronate (V)

A solution of *IV* (0.7 g) in 1,2-dimethoxyethane (20 ml) was diluted with water (10 ml) and, after cooling in an ice bath, aqueous 1 M potassium hydroxide (2.2 ml, ~100% excess required for the saponification of the methyl ester function) was added. Further amount (9 ml, ~100% excess required for the saponification of the *O*-acetyl groups) of the potassium hydroxide solution was added

portionwise after 30 min and the solution was kept at 50—60°C for 1 1/2 hr. The reaction mixture was cooled in ice and deionized with Dowex 50 W (H^+) resin, filtered and concentrated with several additions of water and methanol to remove acetic acid, and ethereal diazomethane was added to the solution of the residue in methanol. Besides the main product *V* (R_F 0.15, solvent *E*) t.l.c. revealed also the presence of two minor components (R_F 0.25, 0.3) which were removed by elution of the crude product from a silica gel column (2.3 × 32 cm) with solvent *E*. The thus obtained chromatographically pure *V* (0.4 g; 81.6%), when crystallized from 96% ethanol and dried at 30°C/2 kPa, showed a double m.p. 136—138, 180—181°C and $[\alpha]_D^{23}$ +53° (*c* 1, water) not changed on recrystallization from the same solvent even when seeded with the sharply melting material (m.p. 180—181°C, $[\alpha]_D^{23}$ +54.5° (*c* 1, water)) obtained by drying the double-melting material at 160—165°C/2 kPa for 30 min.

For $C_{14}H_{24}O_{12}$ (384.33) calculated: 43.75% C, 6.29% H; found: 43.55% C, 6.24% H.

Methyl[methyl 2-O-(β-D-glucopyranosyl)-3,4-O-isopropylidene--α-D-galactopyranosid]uronate (VI)

Compound *III* (1 g) was deacetylated as described for the preparation of *V*, the crude product was allowed to react with ethereal diazomethane and, since t.l.c. (solvent *F*) showed that in addition to *VI* (R_F 0.25) two faster moving minor compounds (R_F 0.4, 0.45) and an olefinic substance (R_F 0.1) were also present, the crude product was purified by column chromatography (35 × 2.5 cm) with solvent *F*. Pure *VI* was obtained as a hygroscopic solid foam which, immediately after drying (40°C/13.3 Pa), showed $[\alpha]_D^{23}$ +56° (*c* 1.04, methanol). Ref. [3] gives $[\alpha]_D^{25}$ +54.4° (*c* 1, methanol) for the amorphous *VI*.

For $C_{17}H_{28}O_{12}$ (424.39) calculated: 48.11% C, 6.65% H; found: 48.37% C, 6.68% H.

Methyl[methyl 2-O-(2,3,4,6-tetra-O-methyl-β-D-glucopyranosyl)--3,4-di-O-methyl-α-D-galactopyranosid]uronate (VII)

A mixture of *V* (0.3 g), silver oxide (4.5 g), and methyl iodide (1.2 ml) in *N,N*-dimethylformamide (10 ml) was vigorously stirred in the dark for 24 hrs. Chloroform (10 ml) was added, the mixture was filtered, the solids washed with chloroform and the filtrate was concentrated at 70°C/2 kPa to remove *N,N*-dimethylformamide. T.l.c. of the residue (solvent *A*) showed the presence of some undermethylated material (R_F 0.1), large proportion of *VII* (R_F 0.25) and a little amount of olefin *X* (R_F 0.4) detectable with both reagents (see above). Separation on a column (26 × 2.6 cm) of silica gel with solvent *A* gave chromatographically pure *X* (17 mg, 5%) and *VII* (0.28 g, 76.5%). Compound *VII*, when crystallized from ether—hexane (1 : 1, twice), melted at 135—136°C and had $[\alpha]_D^{23}$ +66° (*c* 1, chloroform).

For $C_{20}H_{36}O_{12}$ (468.49) calculated: 51.27% C, 7.75% H; found: 51.20% C, 7.80% H.

Olefin *X* had $[\alpha]_D^{23}$ +111 (*c* 1, chloroform) and crystallized immediately when seeded with *X* prepared in the below-described independent manner.

Methyl[methyl 4-deoxy-2-O-(β-D-glucopyranosyl)-β-L-threo--hex-4-enopyranosid]uronate (IX)

Methanolic 1 M sodium methoxide (2 ml) was added to the solution of *III* (1 g) in a mixture of methanol and dry 2,2-dimethoxypropane (15 : 1; 16 ml) and the solution was heated at 50°C for 30 min with the exclusion of atmospheric moisture and carbon dioxide. T.l.c. in solvent *B* then showed the

absence of the starting material (R_F 0.3). The mixture was cooled in an ice bath, deionized with Dowex 50 W (H^+) resin, filtered, ethereal diazomethane was added until a yellow colour persisted, and concentrated. The solid residue (0.6 g; 97%) was homogeneous according to t.l.c. (solvent *E*, R_F 0.35, detection *a* and *b*) and *IX*, after several recrystallizations from methanol, melted at 114—118°C and had $[\alpha]_D^{23}$ +105° (*c* 1.02, water). These values remained unchanged on further recrystallization.

For $C_{14}H_{22}O_{11}$ (366.32) calculated: 45.90% C, 6.05% H; found: 46.0% C, 6.03% H.

Methyl[methyl 4-deoxy-2-O-(2,3,4,6-tetra-O-methyl-β-D-gluco-pyranosyl)-3,4-di-O-methyl-β-L-threo-hex-4-enopyranosid]uronate (X)

Compound *IX* (0.32 g) was methylated with methyl iodide (1.15 ml) and silver oxide (4.3 g) in *N,N*-dimethylformamide (10 ml) in the manner described for the preparation of *VII*. The isolated crude product containing mainly *X* (R_F 0.35; solvent *B*) and some undermethylated material was purified by chromatography on a silica gel column (26 × 2.6 cm) to give pure *X* (0.35 g; 92%) which, when crystallized twice from isopropyl ether—hexane (4:1) melted at 63.5—65.5°C and had $[\alpha]_D^{23}$ +113.8° (*c* 0.97, chloroform).

For $C_{19}H_{32}O_{11}$ (436.45) calculated: 52.28% C, 7.39% H; found: 52.47% C, 7.30% H.

Methyl[methyl 3,4-di-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl--β-D-glucopyranosyl)-α-D-galactopyranosid]uronate (VIII)

a) A mixture of *IV* (0.2 g), dry pyridine (1 ml), and acetic anhydride (2 ml) was left at room temperature for 16 hrs after which time t.l.c. (solvent *D*) showed that the reaction was complete. The solution was poured into ice-cold saturated solution of sodium hydrogen carbonate and, when the excess of acetic anhydride hydrolyzed completely, the product *VIII* (R_F 0.6) was extracted with chloroform. The chloroform solution was washed with 0.5 M sulfuric acid to remove pyridine, sodium hydrogen carbonate solution, water, dried with anhydrous sodium sulfate and concentrated to dryness. The solid residue (0.23 g, ~100%) was crystallized from methyl acetate—ether and after recrystallization from the same solvent *VIII* melted at 177°C and showed $[\alpha]_D^{23}$ +65° (*c* 1, chloroform).

For $C_{26}H_{36}O_{18}$ (636.55) calculated: 49.05% C, 5.70% H; found: 49.02% C, 5.75% H.

b) Acetylation of *V* as described above gave virtually a theoretical yield of material identical in all respects with the above-described substance *VIII*.

Results and discussion

A pseudoaldobiouronic acid derivative of the D-glucose-1→2-D-galactouronic acid type was first synthesized by *Šipoš* and *Bauer* [3, 4]. With the aim to obtain the α-linked compound the cited authors allowed to react 2,3,4,6-tetra-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranose (*I*) with methyl(methyl 3,4-*O*-isopropylidene-α-D-galactopyranosid)uronate (*II*) and obtained a mixture of α- and β-linked oligosaccharides the ratio of which depended largely upon the drying agent used in the reaction. We have repeated the condensation of *I* with *II* under the conditions which gave the highest yield of the β-linked substance [3] and observed, by monitoring the reaction course by t.l.c., that the reaction time applied originally could be substantially reduced. The reaction of *II* with 1.5 mole of

bromide *I* afforded after 48 hrs and chromatography of the crude product a 70% yield of the disaccharide *III*, as compared to 41% after 21 days reported in [3]. The structure of *III* was confirmed by its mass spectrum containing a peak at *m/e* 577 ($[M-15]^+$) from which both the molecular weight of the substance (592) and the presence of the *O*-isopropylidene group could be deduced. The presence of the *O*-isopropylidene group was proved also by the peak at *m/e* 100 [5]. The structure of the neutral cycle was confirmed by the *aA* Series [6, 7], represented by the peaks at *m/e* 331, 271, 229, 211, 169, 127, and 109 [8] dominating in the spectrum.

The synthesis of the title derivative required regeneration of the alcoholic hydroxyl groups in *III* blocked with acid- and base-labile substituents. The removal of the *O*-isopropylidene group was accomplished by mild hydrolysis with dilute acetic acid giving the crystalline methyl[methyl 2-*O*-(2,3,4,6-tetra-*O*--acetyl-β-D-glucopyranosyl)-α-D-galactopyranosid]uronate (*IV*). The selectivity of the hydrolysis, *viz.* the complete substitution of the hexose residue with *O*-acetyl groups was confirmed by mass spectrometry by the presence of the fragments of the *aA* Series in the spectrum of *IV*. As far as the structure of the uronic acid residue was concerned the mass spectrum of *IV* provided no significant information. Compound *IV* was further characterized by its conversion into the crystalline acetate *VIII*. The ions of the *aA* Series present in the mass spectrum of *VIII* were informative again merely with respect to the neutral part of the molecule.

Deacetylation of *IV* with aqueous potassium hydroxide, which removed also the methyl ester function, subsequent treatment with ethereal diazomethane and crystallization from 96% ethanol afforded methyl[methyl 2-*O*-(β-D-glucopyranosyl)-α-D-galactopyranosid]uronate (*V*). The importance of the presence of water in the deacylation of esterified uronic acid derivatives was demonstrated elsewhere in this Series [9]. Acetylation of *V* gave the substance identical with *VIII* obtained by the acetylation of *IV*

Deacetylation of *III* under the conditions of preparation of *V* gave amorphous 3,4-*O*-isopropylidene derivative *VI*, the optical rotation of which was in an excellent agreement with the value reported by the original authors [3]. The molecular weight of *VI* was confirmed by the presence of the $[M-15]^+$ ion peaks in its mass spectrum at *m/e* 409. In addition, the spectrum contained peaks characteristic of the fragmentation of methyl(methyl 3,4-*O*-isopropylidene-hexopyranosid)uronates [5]. The absence of the peaks indicative of the fragmentation of the hexose residue proved that the ionization occurred exclusively on the uronic acid portion of the disaccharide.

It is known that the methylation of hexuronic acid derivatives may be accompanied by β-elimination resulting in the formation of 4,5-unsaturated 4-deoxyhexuronates [10—13]. Since substances of this class are not necessarily the final products of this type of degradation [11, 14], to optimize the yield of the permethylated title product the methylation of *V* had to be conducted under the

possibly mildest conditions. While considerable degradation by β-elimination was observed [11, 17] during methylation by the effective *Hakomori* method [15, 16] model experiments by *Aspinall* and *Barron* [11] showed that no reaction occurred when methyl(methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranosid)uronate was treated with methyl iodide and silver oxide in *N,N*-dimethylformamide (*Kuhn* methylation [18]) for 8 days at room temperature. When *V* was treated with this reagent for 24 hrs a product was formed containing, as shown by t.l.c., besides a small amount of undermethylated material, two chromatographically separable substances. The main product, isolated in a 76.5% yield, was the wanted, crystalline methyl[methyl 2-*O*-(2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl)-3,4-di-*O*-methyl-α-D-galacto-pyranosid]uronate (*VII*). The faster moving by-product, which immediately reduced a dilute acetone solution of potassium permanganate, was methyl[methyl 4-deoxy-2-*O*-(2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl)-3-*O*-methyl-β-L--*threo*-hex-4-enopyranosid]uronate (*X*), formed by β-elimination. The structure *VII* was confirmed by the mass spectrum of the substance for the interpretation of which the principles of the fragmentation of methylated 1→2-linked aldobiouronic acids [7] could be fully applied. The molecular weight was calculated from the aA_1 and bA_1 ion peaks ($M = 219 + 233 + 16 = 468$). The absence of the peak at *m/e* 161 together with the presence of the intense baJ_1 ion peaks at *m/e* 75 proved the 1→2 linkage between the monosaccharide units. The ions abJ_1, represented by the peak at *m/e* 293 in the spectrum, showed that the fully methylated uronic acid was the quasi reducing end unit of the compound.

The structure of olefin *X* could also be deduced from its mass spectrum and was further substantiated by the synthesis of *X* accomplished by a directed β-elimination reaction [19] of *III*. In the mass spectrum of *X* a molecular ion peak was present showing directly the molecular weight of the substance. Retro-Diels—Alder fragmentation gave rise to the ions appearing at *m/e* 292 which again confirmed the 1→2 linkage and, at the same time, the presence of the double bond between C-4 and C-5 of the uronic acid residue [20]. The ions aA_1 and abA_1 appearing in the mass spectrum at *m/e* 219 and 201 proved that the disaccharide olefin *X* consisted of a fully methylated hexose and a 4,5-unsaturated methyl(methylhexopyranosid)uronate.

The synthesis of *X* was based on the fact that the 3,4-*O*-isopropylidene group in methyl *D*-galacturonate can be eliminated under mild conditions [19]. Accordingly, compound *III* containing this arrangement was treated with sodium methoxide in dry methanol containing 2,2-dimethoxypropane as a water-scavenger to give a theoretical yield of crystalline deacetylated olefin *IX*. Compound *IX* gave a reproducible mass spectrum showing that in spite of the presence of several hydroxyl groups in the molecule the substance did not pyrolyze under the conditions of the measurements. The base peak in the spectrum appearing at *m/e* 74 (bH_1 [20]) represented ions which were most probably formed after the

transfer of one of the hydrogen atoms of the hydroxyl groups to the glycosidic oxygen between the monosaccharide units, breakage of this glycosidic linkage and subsequent retro-Diels—Alder fragmentation. Methylation of *IX* under the conditions described in the methylation of *V* yielded a permethylated, crystalline olefin identical in all respects with the by-product *X* of the methylation of *V*.

Taking into account the results reported by *Aspinall et al.* [11] the formation of the olefin *X* as a by-product of Kuhn methylation of *V* was unexpected and may have been caused by the presence of a different substituent at C-4 of the uronic acid moiety. Since the formation of olefins can also be expected to occur during the methylation of other uronic acid-containing substances, the fact that Kuhn methylation may be accompanied by β-elimination should be taken into account in evaluating the results of methylation analysis of related substrates.

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