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Highly selective nitroxyl radical-mediated oxidation of primary alcohol groups in water-soluble glucans

Arjan E.J. de Nooy [a], Arie C. Besemer [a,*], Herman van Bekkum [b]

[a] *TNO Nutrition and Food Research Institute, Department of Biochemistry, Utrechtseweg 48, 3700 AJ Zeist, Netherlands*

[b] *Delft University of Technology, Laboratory of Organic Chemistry and Catalysis, Julianalaan 136, 2628 BL Delft, Netherlands*

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Abstract

With catalytic amounts of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and hypochlorite/bromide as the regenerating oxidant in water, primary alcohol groups in glucans and derivatives thereof were rapidly and completely oxidised. For pyranosides, selectivity was higher than 95% and no side products could be detected with ^{1}H and ^{13}C NMR or with high-performance anion-exchange chromatography (HPAEC). The optimum pH for the reaction was between 10 and 11. The oxidation was found to be first order in TEMPO and Br^-. The oxidation method can be applied to determine the amount of primary alcohol groups in water-soluble glucans; for pullulan, a proportion of 70% and for dextran, a proportion of 3% primary alcohol groups was found.

Keywords: Oxidation; Primary alcohol; Uronic acid preparation; TEMPO oxidation by; Pullulan

1. Introduction

More than half a century ago, it was found by Maurer and Drefahl [1], and Yackel and Kenyon [2] that NO_2 (N_2O_4) preferentially oxidises the primary alcohol groups in carbohydrates to obtain uronic acids. Ever since, oxidation with nitrogen oxides has remained the method of choice for the oxidation of polysaccharides to polyuronic acids. More recently, a modified procedure with in situ generation of nitrogen oxides was described by Painter [3]. High oxidation grades of primary alcohol functions can be obtained, but the reaction is accompanied by substantial degradation of the polymer and by non-selective oxidation which makes a reduction step with $NaBH_4$ necessary [3].

* Corresponding author.

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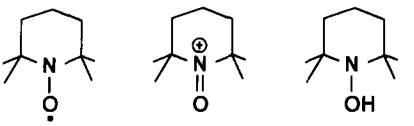
Smaller substrates (monomers, oligomers) can be selectively oxidised with Pt/O_2 [4]. However, this reaction proceeds only sluggishly and with a low degree of oxidation when it is applied to polysaccharides [5] because of the heterogeneous character of the catalyst.

A promising method for the selective oxidation of primary alcohols in the presence of secondary ones originates from the work of Semmelhack et al. [6]. These authors used the stable organic nitroxyl radical 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO, **1**) as a mediator. The actual oxidant [7] is the nitrosonium ion **2**, which can be synthesised from TEMPO by several methods [8]. During the oxidation, **2** is reduced to the hydroxylamine **3**. Nitrosonium ion **2** can be used in a stoichiometric amount [7,9], however, the high chemical reactivity generally hinders its isolation and purification. Fortunately, radical **1** can also be used in a catalytic amount as a mediator [6,10–14], which is in situ oxidised and regenerated by an oxidant.



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Only very recently, the use of organic nitroxyl radicals has been introduced in carbohydrate chemistry for the selective oxidation of primary alcohols [12–14]. In contrast to previous publications on oxidations mediated by nitroxyl radicals, carboxylates were obtained instead of aldehydes. In a patent application, Casciani et al. [12] described the oxidation of an alkyl polyglucoside in water; a catalytic amount of TEMPO was used and the oxidant was regenerated with $OCl^-/HOCl$ at pH 8.6. Yield and selectivity of the reaction were not mentioned. Davis and Flitsch [13] used TEMPO in a two-phase system for the selective oxidation of partially protected monosaccharide derivatives. These authors anticipated the use of TEMPO for the oxidation of polysaccharides.

Independently, we have been working on the TEMPO-mediated oxidation of various water-soluble polysaccharides with hypochlorite/bromide as the regenerating oxidant and water as the solvent. Since TEMPO is soluble in water, a homogeneous reaction system is obtained. In a preliminary communication [14], the oxidation of potato starch and dahlia inulin using this reaction system was described. It appeared that the reaction was more selective for pyranosides than for furanosides. Therefore, an investigation was done towards optimization of this system with some water-soluble glucans and glucose derivatives, the results of which are presented in the sequel.

2. Results and discussion

TEMPO-mediated carbohydrate oxidation, performed under the general conditions described in the Methods section, was highly selective for the substrates studied.

Table 1
Results of the TEMPO-mediated oxidation of different substrates

Substrate [a]	pH	Temperature (°C)	Time (min) [b]	Yield (%) [c]	Selectivity (%) [d]
Methyl α-D-glucopyranoside	10	2	55	n.i. [c]	> 95
Methyl ß-D-glucopyranoside	10	2	35	n.i.	> 95
α,α-Trehalose	10	2	50	n.i.	> 95
Potato starch	10.8	2	80	98	> 95
Amylodextrin	10	20	45	88 [f]	> 90
Pullulan	10.5	2	70	95	> 95

[a] Conditions as described in the Methods section.
[b] Time necessary for the consumption of 1 mmol OH^-/mmol primary alcohol.
[c] The yield was calculated with the molecular weight of the oxidised products as the sodium salt of polyuronic acids.
[d] Percentage of oxidation of primary alcohols with respect to total oxidation.
[e] Not isolated.
[f] Some loss occurred due to only partial precipitation with ethanol.

Primary alcohols were rapidly and completely oxidised to carboxylate functions (Table 1). No other oxidation products could be detected with ^1H and ^{13}C NMR, which is demonstrated by the ^{13}C NMR spectra of oxidised potato starch and pullulan (Fig. 1, NMR data are given in Table 2). Furthermore, the *m*-hydroxybiphenyl method [16] for quantitative detection of uronic acids showed a linear relation between consumption of OH^- and formation of uronate. The substrates were completely converted into uronates after one mmol OH^- per mmol primary alcohol was consumed (Fig. 2). Subsequently, a slow background reaction proceeded as evidenced by the consumption of ca. 0.25 mmol base per hour. This is to be expected, because it is well-known that hypohalite under

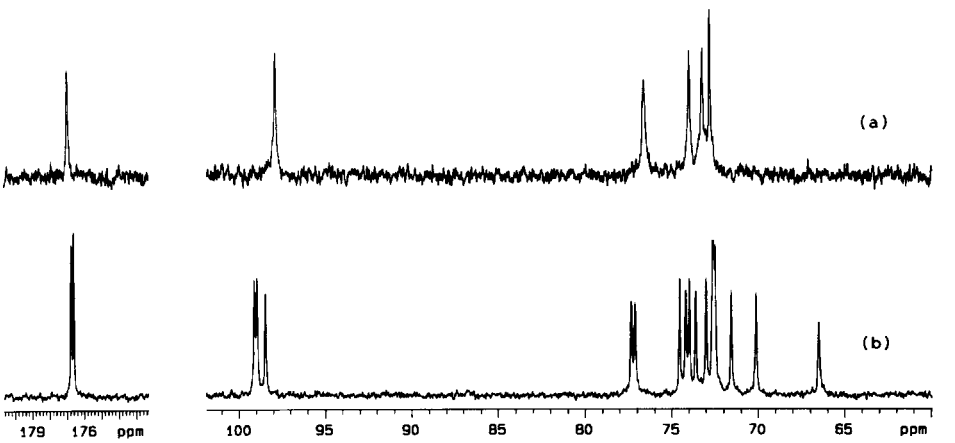


Fig. 1. ^{13}C NMR spectra of oxidised potato starch (a) and oxidised pullulan (b). Note that the 18 peaks of the three different anhydroglucose units of oxidised pullulan are shown. Two C-6 primary hydroxyl carbon peaks in pullulan between 61 and 62 ppm have disappeared and two peaks appeared in the carboxylic acid region. (For peak assignment see Table 2.)

Table 2
^{13}C NMR and ^{1}H NMR chemical shifts (ppm) and $^{1}H-^{1}H$ coupling constants (Hz) of the oxidised products (sodium salts of the uronic acids)

	M α-g [a]	M ß-g [b]	α,α-T [c]	P.st. [d]	A.d. [e]	Pullulan [f]		
						→ 6g1 → 4	→ 4g1 → 4	→ 4g1 → 6
C-1	100.4 [g]	104.2	94.8	97.9	98.0	99.1	98.5	99.0
C-2	72.1	74.1	72.0	72.8	72.8	72.63	72.57	72.48
C-3	74.0	76.7	73.6	74.0	73.9	74.0	74.2	74.5
C-4	73.1	73.0	73.2	76.6	77.0	70.1	77.3	77.1
C-5	73.0	77.1	73.5	73.2	73.2	71.5	73.6	73.0
C-6	177.7	177.0	177.7	177.0	177.0	66.5	176.8 [h]	176.6 [h]
C_{OMe}	56.4	58.7						
H-1	4.89	4.53	5.36	5.56	5.59	5.49	5.54	4.93
H-2	3.67	3.43	3.88	3.49	3.55			
H-3	3.75	3.66	4.04	3.94	3.99			
H-4	3.56	3.62	3.70	3.66	3.71			
H-5	3.96	3.87	4.25	4.02	4.10			
H_{OMe}	3.48	3.69						
$J_{H-1,H-2}$	3.4	7.9	3.8	3.1	3.0	3.4	2.7	2.7
$J_{H-2,H-3}$	9.5	8.2	9.9					
$J_{H-3,H-4}$	9.3	9.0	9.1					
$J_{H-4,H-5}$	9.9	9.5	10.1					

[a] Methyl α-D-glucopyranosiduronate.
[b] Methyl ß-D-glucopyranosiduronate; the ^{13}C NMR assignments are in agreement with those obtained by Gorin and Mazurek [15] for methyl α-D-glucopyranosiduronic acid and methyl ß-D-glucopyranosiduronic acid. Only the shift of C-5 was found to be 1–1.5 ppm higher due to the effect of the dissociated ion of the COOH.
[c] C-6, C-6′ oxidised α,α-trehalose.
[d] C-6 oxidised potato starch.
[e] C-6 oxidised amylodextrin.
[f] C-6, C-6′ oxidised pullulan.
[g] TPS in D_2O was used as an external reference. $\delta_{TPS} = 0$ ppm for ^{1}H spectra and $\delta_{TPS} = -1.8$ ppm for ^{13}C spectra. All data were affirmed with 2D-COSY and HC-correlation spectroscopy.
[h] Assignments may be reversed.

basic conditions oxidises secondary alcohols [17]. The high selectivity was affirmed with HPAEC; after oxidation of the smaller glucose derivatives, only one product could be detected. Methyl β-D-glucopyranoside reacted substantially faster than its α isomer (Table 1); this remains to be explained.

The selectivity for the primary alcohol group oxidation was generally at least 95%. The amylodextrins, which have, on average, one reducing end-group per 25 anhydroglucose units, were oxidised somewhat less selectively. From an experiment with D-glucose as the substrate with excess hypohalite, it appeared that oxidation proceeded after 2 mol OH^-/mol D-glucose was consumed. This indicates that the reducing terminal glucose unit in amylodextrins is oxidised further to smaller fragments. Also, the higher reaction temperature applied, because of the limited solubility of amylodextrins, might have a negative effect on the selectivity, which was still over 90% (Table 1). In the oxidation of furanosides like inulin [14] and sucrose [18], the consumption of base was found to proceed more rapidly than with pyranosides after the theoretical amount was added.

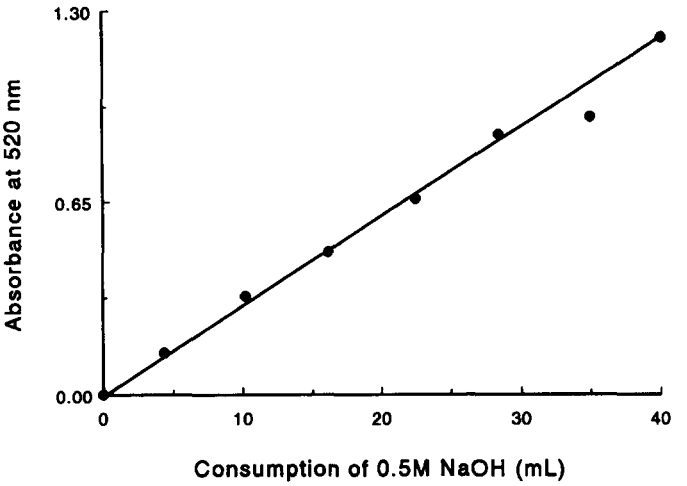


Fig. 2. Formation of uronate as measured with the *m*-hydroxybiphenyl method during the oxidation of methyl α-D-glucopyranoside. Reference solutions of the sodium salt of D-glucuronic acid (absorbance 1.24) and D-galacturonic acid (absorbance 1.20) were comparable with the value obtained for the completely oxidised product (after consumption of 20 mmol OH^- the absorbance was 1.21).

Apparently, this oxidation method when applied to carbohydrates, is most selective for pyranosides. Thus, α,α-trehalose was oxidised essentially quantitatively to the 6,6′-di-carboxy-system.

As is shown in Fig. 1 pullulan, a linear water-soluble polymer consisting of the repeating trimer [→ 6)-α-D-Glc*p*-(1 → 4)-α-D-Glc*p*-(1 → 4)-α-D-Glc*p*-(1 →] with small portions of three consecutive (1 → 4) linkages, was also oxidised very selectively. In agreement with its structure, the consumption was 0.70 mmol NaOH per mmol anhydroglucose unit. So, it is possible to use this oxidation for the determination of the proportion of primary alcohols. With dextran, a (1 → 6)-α-D-glucan with varying proportions of other linkage types as a substrate, ca. 3 primary alcohol groups per 100 anhydroglucose units were oxidised, based on extrapolation of the background reaction to time = 0 (Fig. 3). To determine whether this background reaction was a TEMPO-mediated non-selective oxidation or a non-selective oxidation due to the presence of hypohalite, a control experiment without TEMPO was done (Fig. 3). As the slopes of the graphs are comparable, it seems that most non-selective oxidation in the reaction system is due to hypohalite oxidation.

In contrast to the oxidation of the polymers, oxidation of methyl α-D-glucopyranoside was first order in substrate after an induction period. In the following discussion, reaction rates are relative first order *k*-values with respect to methyl α-D-glucopyranoside. They are corrected for the increase in volume due to the addition of NaOH. All relative reaction rates are average values of at least 3 experiments and the relative reaction rate of the oxidation under conditions as described in the Methods section is taken as 1.

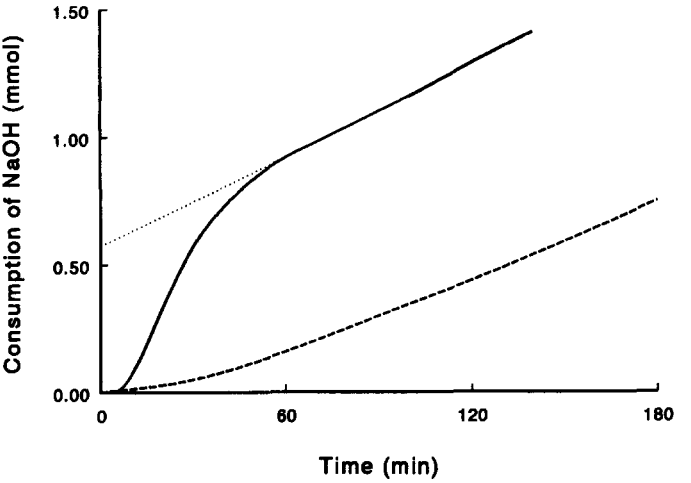


Fig. 3. Oxidation of dextran (20 mmol anhydroglucose units) according to the conditions described in the Methods section with TEMPO (-) and without TEMPO (–) added to the mixture. From extrapolation of the background reaction of the TEMPO-mediated oxidation to t = 0 the proportion of primary alcohol groups can be calculated (0.6 mmol/20 mmol anhydroglucose units, 3% primary alcohol groups).

The influence of the pH on the reaction rate of methyl α-D-glucopyranoside was considerable. Although in previous publications [11–13] on the oxidation of primary alcohols with a TEMPO/hypohalite system, the pH was maintained between 8.5 and 9.5, we found a substantial increase in oxidation rate at a higher pH (Fig. 4). This is advantageous because non-selective hypohalite oxidation of sugars has a higher reaction rate at pH < 9.5 [17]. From investigations by Golubev et al. [19] and Semmelhack et al.

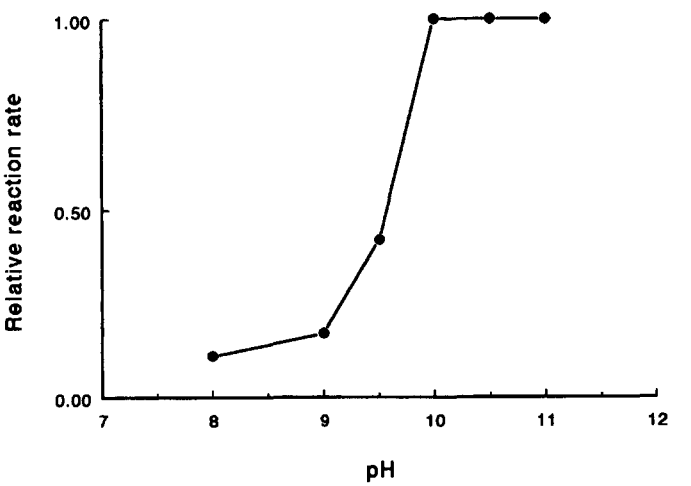


Fig. 4. Influence of the pH on the rate of oxidation of methyl α-D-glucopyranoside.

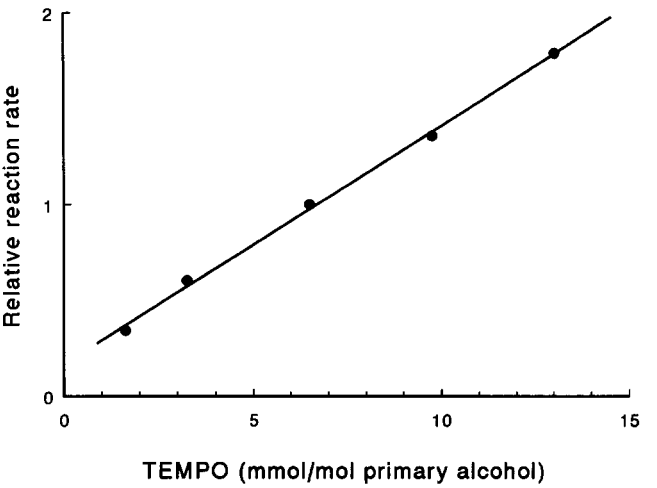
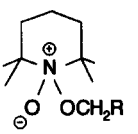


Fig. 5. Effect of the TEMPO concentration on the rate of oxidation of methyl α-D-glucopyranoside.

[20], it seems plausible to assume an intermediate like **4** in the rate limiting step. This intermediate is expected to be formed more easily at high pH, due to the loss of a proton in **4** which could explain the increase of reaction rate at higher pH. However, a change in pH influences several variables in the reaction system. For example, conversion of hypochlorite to hypobromite becomes slower at high pH [21] and might become rate limiting, which makes an interpretation of the observed pH dependence of the reaction difficult. Furthermore, from a series of experiments with potato starch as the substrate, it appeared that at pH 10.5–11 the reaction proceeded faster than at pH 10. When the pH was raised above 11 the reaction was retarded again.



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A linear relation was found between the rate of oxidation of methyl α-D-glucopyranoside and the concentration of TEMPO (Fig. 5) and of NaBr (Fig. 6). When no NaBr was added to the mixture, the reaction rate decreased significantly which shows that OBr^-/HOBr is a more reactive oxidant than OCl^- (at pH 10 more than 90% of hypobromous acid and more than 99% of hypochlorous acid is dissociated). In agreement with the results of Anelli et al. [11], we found that the same reaction rates can be obtained by decreasing the concentration of TEMPO and increasing the concentration of Br^-. However, due to the higher concentration of hypobromite during the reaction, this will decrease the selectivity for primary alcohol oxidation. In contrast to the results from these authors, we found that an increase in temperature also increased the reaction rate. It is well-known, however, that the reaction rate of non-selective oxidation with

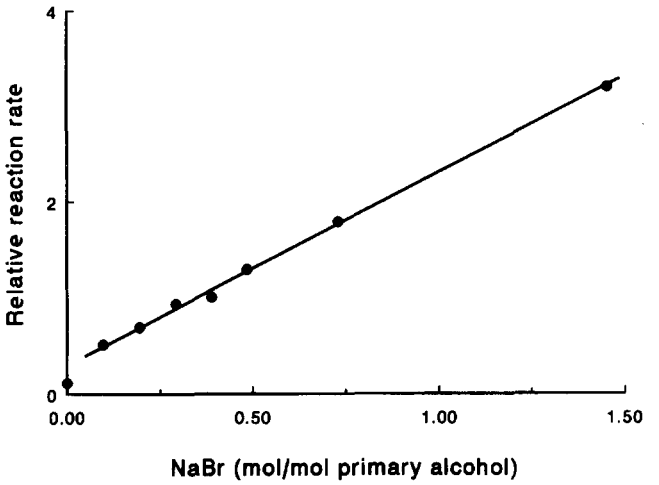

Fig. 6. Influence of the NaBr concentration on the reaction rate of methyl α-D-glucopyranoside.

hypohalite also increases with temperature [17]. A detailed investigation of the kinetics of the TEMPO system is in progress.

3. Experimental

Materials.—Water-soluble potato starch was a gift from Avebe (Veendam, Netherlands). Linear dextrins (amylodextrins, average molecular weight 4000) were prepared from waxy maize starch by enzymatic hydrolysis. Dextran (average molecular weight 40 000) was acquired from Pharmacia (Uppsala, Sweden) and pullulan was acquired from Hayashibara (Okayama, Japan). Methyl α-D-glucopyranoside, methyl ß-D-glucopyranoside, α,α-trehalose, and TEMPO were obtained from Sigma (St. Louis, MO, USA). A sodium hypochlorite solution (ca. 15% active chlorine) was a gift from AKZO-Nobel (Arnhem, Netherlands). All other chemicals were analytical-grade commercial products and were used without prior purification.

Methods.—Unless otherwise stated, all experiments were done under the following conditions: the carbohydrate (20 mmol primary alcohol), TEMPO (0.13 mmol, 0.02 g), and NaBr (7.8 mmol, 0.8 g) were dissolved in water (500 mL). A 15% sodium hypochlorite solution (2.2 mmol sodium hypochlorite/mmol primary alcohol, 10% excess) was adjusted to the desired pH by adding aq 4 M HCl. Both solutions were brought to the desired temperature; the reaction was conducted at 2 ± 1°C, only the linear dextrins were oxidised at room temperature, and added at once to each other. The pH was controlled with a pH-stat (pH 10, only potato starch and pullulan were oxidised at higher pH) by adding 0.5 M NaOH. In this way, the formation of acid during the reaction was monitored. The uronate concentration was followed with the *m*-hydroxybiphenyl [16] colorimetric method, by taking aliquots during the reaction.

When the oxidation was finished, the reaction was quenched by adding 96% EtOH (10 mL) and the pH was adjusted to 7 by adding aq 4 M HCl. The oxidised polysaccharides were isolated by adding EtOH until a white precipitate formed. The precipitate was centrifuged and washed several times with 70:30 EtOH–water. The product was dried under reduced pressure at 50°C. The oxidised glucose derivatives were dried under reduced pressure and were analysed without further purification.

Analysis.—NMR spectra were recorded on a Varian UNITY-400 spectrometer operating at a proton NMR frequency of 400 MHz and a carbon NMR frequency of 100 MHz. Carbon spectra were recorded in the gated decoupling mode. All oxidised products were dissolved in D_2O. Peak assignment of the ^{1}H and ^{13}C NMR spectra of all products was achieved with 2D-COSY and HC-correlation spectroscopy.

HPAEC-analysis was performed with a Dionex DX-300 ion chromatograph. A CarboPac PA-1 column equipped with a CarboPac PA-1 guard column was kept in a 20°C water bath. Amperometric detection was performed with a Dionex pulsed electrochemical detector (PED) in the pulsed amperometry mode. The PED detector was equipped with a gold working electrode and an Ag/AgCl reference electrode. Eluent: 0–5 min; 100 mM NaOH, 5–10 min; linear gradient to 80% 100 mM NaOH and 20% 100 mM NaOH + 500 mM NaAc, > 10 min 80% 100 mM NaOH and 20% 100 mM NaOH + 500 mM NaAc.

Acknowledgements

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