

Response Surface Investigation and Optimisation of an Etherification Reaction of a Compound Containing Several Hydroxylic Groups

Hans-René Bjørsvik^{†,a} and Audun Aukrust^b

Nycomed Imaging AS, ^aDepartment of Process Chemistry and ^bDepartment of Development Chemistry, P.O.Box 4220 Torshov, N-0401 Oslo, Norway

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A synthetic procedure for an ether by dehydration of a compound containing several hydroxylic groups has been developed. By using response surface design and partial least-squares regression (PLSR modelling) a high yielding procedure has been established.

In spite of the presence of many hydroxylic groups, both primary and secondary in the treated substrate, the PLSR model predicted successfully the experimental conditions to achieve a surprisingly high yielding synthetic procedure for the ether by dehydration of the primary hydroxylic groups.

The chemical information obtained through the present study may be of general interest when a preparative etherification reaction from an alcohol is required.

3,3',5,5'-Tetrakis(2,3-dihydroxypropylcarbonyl)-2,2',4,4',6,6'-hexaiodo-*N,N'*-(2-hydroxypropane-1,3-diyl) diacetanilide **2**, Scheme 1, is interesting as a general X-ray contrast agent for medical imaging.^{1,2} By the nature of this application, impurities (by-products) determined in bulk substance must be isolated, their molecular structure elucidated, and their biological effects (toxicology) need to be explored.

The substance **2** can be reached by many synthetic paths. During the last step in one method, deacetylation of the *O*-acetylated compound **1** is performed by treatment with aqueous sulfuric acid at elevated temperatures overnight. Under these conditions, small quantities of a by-product, 0.16% of the reaction mixture, are obtained. Hence, in order to perform the biological studies it was necessary to obtain about 2 g of this compound, determined to be the ether **3**. Owing to the small quantities of the ether in the 'standard' reaction mixture, a high yielding synthetic procedure was required.

The side-reaction yielding the compound **3** is a dehydration of a primary alcohol to form the corresponding ether.³ This reaction proceeds by initial protonation of the hydroxylic group of the alcohol by the acid to yield an

oxonium salt. The oxonium salt then undergoes a nucleophilic attack by an alcohol molecule during formation of the ether.

However, this reaction may proceed on both primary, secondary and tertiary alcohols. In the present case, many reaction products are possible owing to the presence of both secondary and primary hydroxylic groups in the substrate.

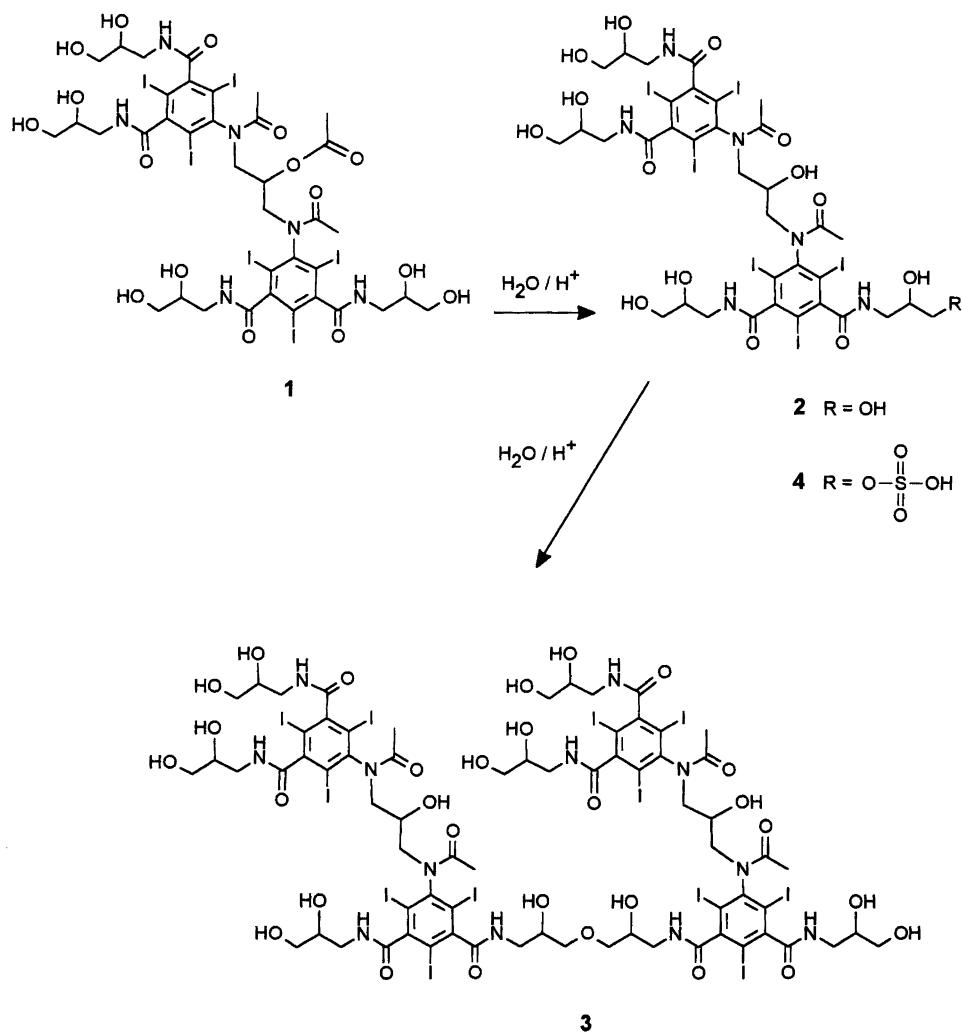
Based upon some *a priori* knowledge³ and some introductory screening experiments, a subsequent response surface design and modelling study was carried out to obtain a model including linear terms, two-factor interactions and quadratic terms. From this empirical model, a high yielding etherification reaction under conditions resembling pyrolysis, was established.

Sendersens⁴ has pointed out that it is very important to select carefully the synthetic conditions, catalyst, etc. in order to obtain a successful synthesis of an ether. Owing to the multivariate study of the experimental variables, the present procedure may be of interest when a synthetic procedure for an ether from an alcohol is required.

Methods and results

Experimental variables and domains. The objectives of the present study were (i) to determine which variables in-

[†] To whom correspondence should be addressed.
Present address: EuroVanillin, P.O.Box 330, N-1701 Sarpsborg, Norway.



Scheme 1.

fluence the formation of the ether **3**, (ii) to determine the detailed conditions of these variables which give maximum yield, and (iii) to determine other possible intermediates or by-products. In order to find a more suitable domain for the synthetic conditions to obtain the ether **3**, some introductory experiments were carried out. The cube plot in Fig. 1 summarizes these conditions and results. Based upon these observations, a central composite design was carried out: x_1 : concentration of sulfuric acid [mol l^{-1}], x_2 : reaction temperature [$^{\circ}\text{C}$], x_3 : substrate concentration [g ml^{-1}], and x_4 : reaction time [h]. The explored domain is specified in Table 1.

A model, eqn. (1), which relates the response, η [$=f(y)$], to the experimental variables x_1 – x_4 included both linear and non-linear effects was used.

$$\eta = \beta_0 + \sum_{k=1}^K \beta_k x_k + \sum_{k=1}^{K-1} \sum_{l=2}^K \beta_{kl} x_k x_l + \sum_{k=1}^K \beta_{kk} x_k^2 + \varepsilon \quad (1)$$

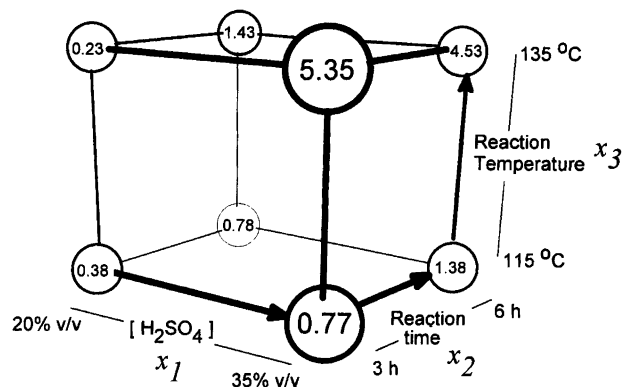


Fig. 1. Experimental design including parameter settings with the corresponding response measured on HPLC. A preliminary modelling on these results yielded the following model for the ether **3**: $y = 1.92 + 1.09x_1 + 1.09x_3 + 0.84x_1x_3 - 0.22x_1x_2x_3$.

Table 1. Experimental design and results from HPLC analysis.

#	Experimental variables ^a				Responses ^b			
	x_1	x_2	x_3	x_4	y_1	y_2	y_3	y_r
1	-1	-1	-1	-1	2.08	83.21	3.82	10.89
2	+1	-1	-1	-1	12.65	49.59	25.34	12.42
3	-1	+1	-1	-1	2.25	82.05	12.96	2.77
4	+1	+1	-1	-1	20.20	36.66	19.66	23.48
5	-1	-1	+1	-1	1.37	83.48	14.22	0.93
6	+1	-1	+1	-1	14.00	47.81	27.40	10.79
7	-1	+1	+1	-1	1.07	83.62	13.87	1.44
8	+1	+1	+1	-1	8.82	43.13	23.20	24.85
9	-1	-1	-1	+1	3.34	81.09	13.85	1.72
10	+1	-1	-1	+1	10.79	47.13	25.35	16.43
11	-1	+1	-1	+1	3.19	80.04	13.23	3.54
12	+1	+1	-1	+1	19.28	28.61	13.48	36.63
13	-1	-1	+1	+1	1.07	83.18	14.51	1.24
14	+1	-1	+1	+1	11.69	45.83	32.17	10.31
15	-1	+1	+1	+1	1.34	83.47	12.37	2.82
16	+1	+1	+1	+1	10.12	35.67	19.69	34.52
17	-2	0	0	0	0.00	93.96	5.58	0.46
18	+2	0	0	0	46.30	12.36	12.91	28.43
19	0	-2	0	0	3.59	73.40	21.30	1.71
20	0	+2	0	0	6.93	67.89	17.90	7.28
21	0	0	-2	0	3.69	69.41	17.78	9.12
22	0	0	+2	0	0.13	73.21	20.02	6.64
23	0	0	0	-2	2.33	72.68	20.39	4.60
24	0	0	0	+2	8.00	69.86	9.21	12.93
25	0	0	0	0	6.35	63.54	24.60	5.51
26	0	0	0	0	4.72	65.94	23.93	5.41
27	0	0	0	0	3.29	69.34	21.55	5.82
28	0	0	0	0	2.31	74.43	14.92	8.34

^a Experimental variables: x_k (definition levels) [-2, -1, 0, +1, +2]; x_1 concentration of H_2SO_4 mol l^{-1} [2.00, 3.75, 5.50, 7.25, 9.00]; x_2 reaction temperature $^{\circ}C$ [95, 105, 115, 125, 135]; x_3 substrate concentration g ml^{-1} [0.20, 0.30, 0.40, 0.50, 0.60]; x_4 reaction time h [2, 3, 4, 5, 6]. ^b y_1 : HPLC area% of the ether **3**; y_2 : HPLC area% of unchanged substrate **2**; y_3 : HPLC area% of a non-structure-elucidated by-product (assumed to be the sulfate ester **4**); y_r : HPLC area% sum of other by-products ($y_r = 100 - \sum_{i=1}^3 y_i$).

However, a logarithmic transformation, eqn. (2), of the response y was necessary in order to achieve a satisfactory adaptation $y = F(x_1, x_2, x_3, x_4)$. The methods and

$$\eta = \log \left(\frac{y + 1}{100 - y} \right) \quad (2)$$

details of the response surface modelling (RSM) is well described in the literature.⁵⁻⁷

The design matrix and yields obtained in the experimental runs are summarized in Table 1. From these data, the response surface model parameters, the regression coefficients in eqn. (1) were computed using the PLSR method.^{8,9} A three principal component model ($A=3$) was determined to describe 80% ($= 63.9 + 14.8 + 1.3$) of the variance in y_1 . The influencing variables were determined using the cumulative normal probability plot¹⁰ shown in Fig. 2(a).

With respect to obtaining a high yield of the ether **3**, based upon this model, one can conclude that it is beneficial to have (i) a short reaction time, (ii) a high con-

centration of sulfuric acid, (iii) a high reaction temperature, and (iv) a low substrate concentration. In addition to these general considerations, some two-factor interactions and quadratic effects were also determined to have significant influence on the response y_1 (the outcome of **3**).

The final model describing the yield of the ether **3** is given in eqns. (3) and (4). The model in eqns. (3) and (4)

$$\eta_1 = -1.202 + 0.410x_1 - 0.116x_3 - 0.024x_1x_4 - 0.050x_2x_3 - 0.013x_3x_4 + 0.069x_1^2 - 0.082x_3^2 \quad (3)$$

$$y_1 = \frac{10^{(\eta_1 + 2)} - 1}{10^{\eta_1} + 1} \quad (4)$$

was used to simulate the response surfaces in Fig. 3 as well as to predict the results for the experiments carried out. The statistical products indicate an acceptable model: the root mean squares of error prediction, RMSEP = 1.721 and the multiple correlation coefficient, $R^2 = 0.876$ and slope = 0.921 when plotting predicted versus observed.

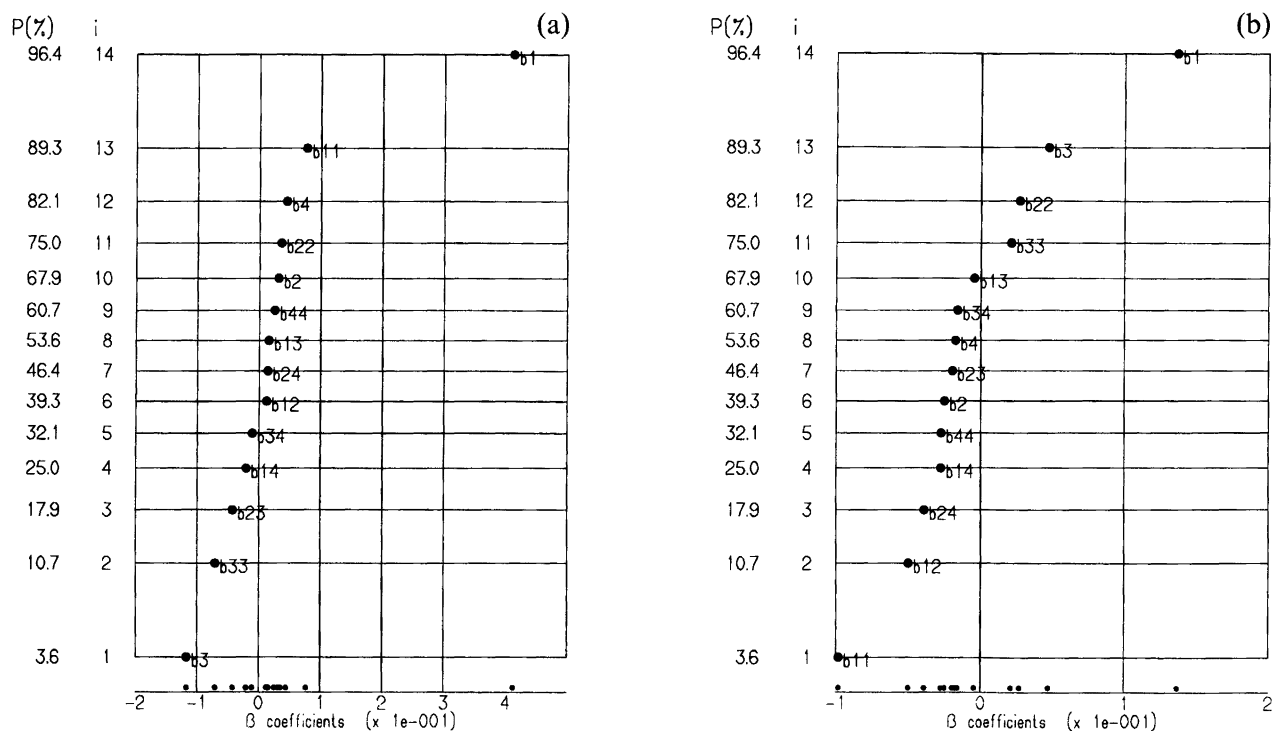


Fig. 2. Normal probability plot of the estimated regression coefficients: (a) model for the ether **3**; (b) model for the assumed product **4** (sulfate ester).

Conditions for the ether procedure. The contour projections of the response surface from the obtained model in eqns. (3) and (4) are shown in Fig. 3. These projections show that a high yield for the ether **3** was to be expected under the following conditions. The substrate **2** (2.0 g, 1.29 mmol) was transferred to a 50 ml flask containing 10 ml sulfuric acid (9.0 M). The flask was immersed in an oil bath at the set temperature (145 °C). The reaction mixture was refluxed under a nitrogen atmosphere, and samples were withdrawn at reaction times of 1.5 h and 2 h, and analysed by HPLC. The experimental results with corresponding predictions using eqns. (3) and (4) are given in Table 2. These results show the explanatory power for the model in eqns. (3) and (4).

Another by-product. In Table 1, the response y_3 shows the variation of a compound assumed to be the sulfate ester

Table 2. Experimental and calculated results from a developed and optimized synthetic procedure.

x_4 Reaction time ^a	Experimental		Predicted
	2 (%)	3 (%)	3 (%)
1.5 h (-2.5)	9.1	51.0	52.9
2.0 h (-2)	0.0	51.2	53.1

^a Companion reaction conditions: x_1 concentration of H_2SO_4 : 9.00 mol l⁻¹ (+2); x_2 reaction temperature 145 °C (+3); x_3 substrate concentration 0.20 g ml⁻¹ (-2).

4. A similar modelling as described for response y_1 above was also carried out for response y_3 . The cumulative normal probability plot in Fig. 2(b) shows the model for the assumed product, the sulfate ester **4**. Interpretation of this plot yields the model given in eqns. (5) and (6). This

$$\eta_3 = -0.633 + 0.133x_1 + 0.046x_3 - 0.049x_1x_2 - 0.038x_2x_4 - 0.096x_1^2 + 0.026x_2^2 + 0.021x_3^2 \quad (5)$$

$$y_3 = \frac{10^{(\eta_3 + 2)} - 1}{10^{\eta_3} + 1} \quad (6)$$

model predicts a high yield using: (i) a high sulfuric acid concentration, (ii) a moderate reaction temperature, (iii) a high substrate (**2**) concentration, and (iv) a long reaction time.

Discussion and conclusion

Chemical interpretation of the regression coefficients of the model. Interpretation of the variable 'reaction time' shows that it is beneficial to apply a short reaction time. This may be due to the fact that the substrate contains more than one primary hydroxylic (and secondary) group which can participate in the etherification reaction during the formation of polyethers. The probability for this increases when the amount of the ether **3** increases. Hence, a long reaction time will imply a decreased final outcome of the desired ether **3**.

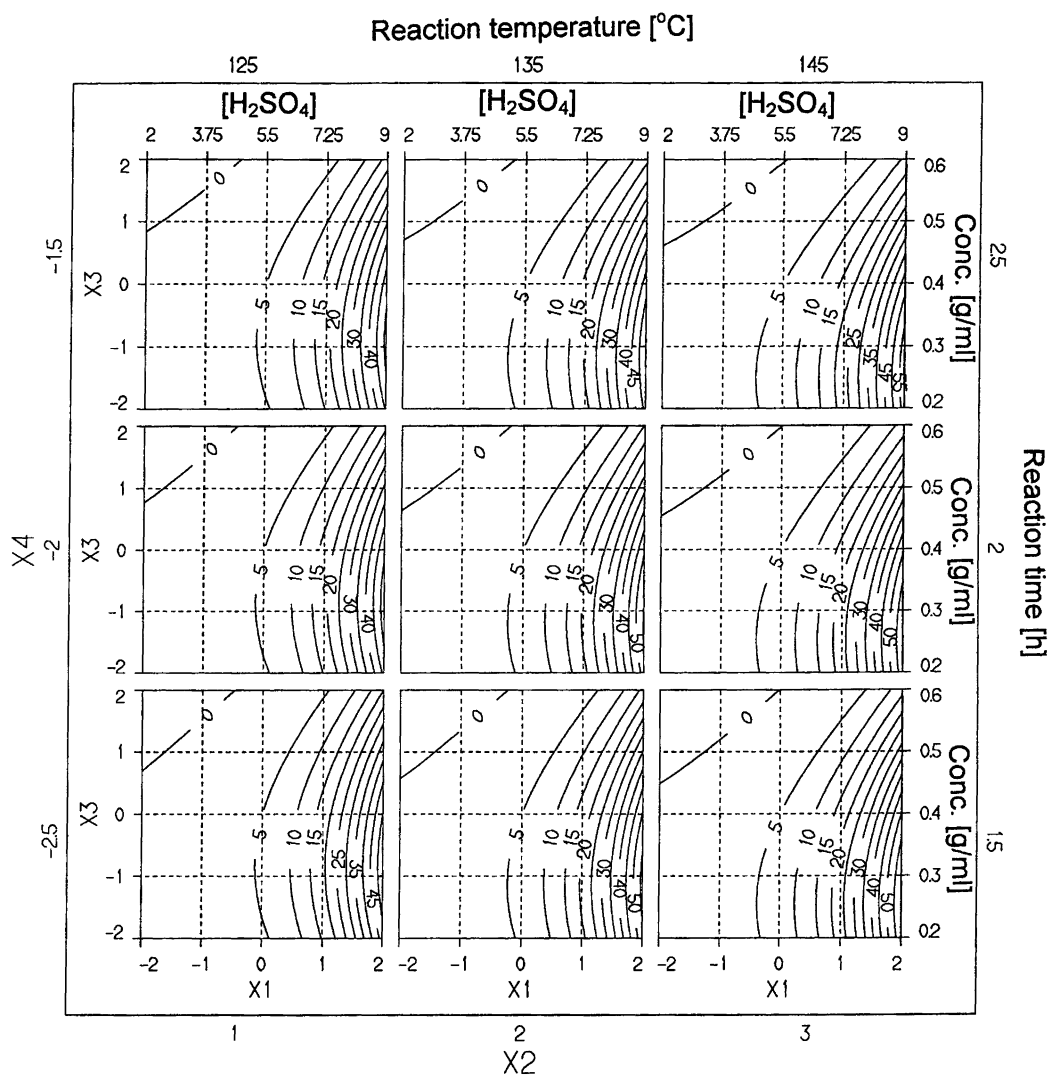


Fig. 3. Response surface projections of eqns. (3) and (4). The contour lines show the yield of the ether **3** when the four experimental variables x_1 concentration of H_2SO_4 mol l^{-1} , x_2 reaction temperature $^\circ\text{C}$, x_3 substrate concentration g ml^{-1} , and x_4 reaction time h are varied. To read the plot, the large frame shows the variation in reaction temperature (x_2) and reaction time (x_4). In this frame, nine subplots showing the contour of the response surface when the experimental variables concentration of H_2SO_4 (x_1) and substrate concentration (x_3) are varied. The subplot in the center right of the large frame, i.e., $x_2 = +3$ and $x_4 = -2$ and the varying of x_1 and x_3 , is used to predict the conditions for the etherification procedure.

It is known that the hydro-dehalogenation (or dehalogenation) reaction occurs catalysed by a Friedel-Craft mechanism. In such reactions, aryl-iodines are the species most easily cleaved. The iodine, which in an acidic environment acts as a catalyst for the cleavage of the ether-linkage,¹¹ is present in this case as the tri-iodine aryl moiety. Hence, a cleavage of the ether linkage may well occur. The formation of the iodine may be promoted by prolonged extreme conditions.

The model shows, in accordance with the literature,³ that it is beneficial to use a high concentration of sulfuric acid. The etherification reaction is in fact a dehydration of an alcohol. Such reactions are promoted by more concentrated sulfuric acid. However, too high a concentra-

tion can lead to degradation of the ether as well as of the substrate. An overview of the amount of such product is given by the variable y , in Table 1. The variable y , is the sum of substances other than **2**, **3**, and a not structure-elucidated by-product, which nevertheless is assumed to be the sulfate ester **4** (Scheme 1).

The observed effect of the reaction temperature is also in accordance with the literature.³ An elevated temperature will increase the proton activity which can then promote the protonation of the alcoholic hydroxylic group of the substrate.

In Table 2, validation experiments are described including both experimentally obtained and predicted results using eqns. (3) and (4). These results show the

explanatory power for the model in eqns. (3) and (4). As the table shows, the reaction proceeds after a reaction time of 1.5 h. However, the yield of the ether **3** does not increase. This could be due to polyether formation.

Concluding remarks. This work has shown how multivariate methods can be applied in order to obtain information regarding the different experimental variables of a synthetic procedure. The model parameters, the regression coefficients, are applied as tools to interpret the different effects observed in the synthetic procedure. Finally, the model was used to improve the outcome of the originally very low yielding synthetic organic reaction.

From an organic chemist's point of view, investigating the optimization of a reaction that gives only 0.16% yield seems rather futile. However, on the basis of the results from the present synthetic problem, one can surmise that many organic syntheses have been discarded based on incorrect assumptions caused by univariate thinking. This is due to lack of strategies and poor experimental designs not able to generate vital information about the chemistry for a certain synthetic reaction. This becomes especially important when variable interactions and/or quadratic effects have significant influence in the model which describes the synthetic reaction.

Experimental

The synthetic procedure. The substrate **2** (2–6 g, 1.29–3.87 mmol) (Nycomed) was transferred to a 50 ml flask containing 10 ml sulfuric acid (2–9 M) (Merck). The flask was immersed in an oil bath at the set temperature (95–135°C) and the solution was refluxed under a nitrogen atmosphere for the set reaction time (2–6 h).

From the reaction mixture, a sample (4.0 ml) was cooled and quenched by addition of 5 M NaOH (EKA Nobel) to neutralize the sulfuric acid. The solution obtained was diluted with purified water and the sample (2.5 mg ml⁻¹) analysed by HPLC.

The complete experimental program with conditions and HPLC results are summarized in Table 1.

HPLC analysis. The samples were analysed by HPLC using an internal standard to determine the amount of the ether **3** and unchanged substrate **2**. The internal standard was obtained from preparative HPLC and characterized by NMR and mass spectrometry (see below).

An autoinjector with variable loop (injection volume 10–20 µl), and a gradient pump were used. A UV-detector with optical path length 10 mm, operating at $\lambda = 254$ nm with response time 0.5 µs was used. A stainless steel column was used, Brownlee Labs, cat. No. AS-5A (L 250 mm, ID 4.6 mm) with amino-propylsilyl silica gel with particle size 5 µm as packing material.

Two eluents, A and B were used, where eluent A was prepared by mixing 500 ml of acetonitrile (Merck) and

500 ml of purified water. Eluent B was pure acetonitrile. The eluents were degassed by sonification prior to use.

The mobile phase delivery system with a flow rate 1.0 ml min⁻¹ was as follows. Equilibration of 30% of eluent A for 15 min, gradient pumping 30–50% of eluent A for 19 min, then gradient pumping 50–100% of eluent A for 30 min. The column was purged with 100% of eluent A for 30 min.

NMR and MS equipment and analysis. The NMR spectra were recorded with a VARIAN VXR 300S NMR instrument equipped with a 5 mm broadband probe. Ether **3**: ¹H NMR (DMSO-*d*₆): δ 8.9–8.0, 5.0–4.4, 4.4–2.7, 4.0–3.8, 2.22, 1.78. ¹³C NMR (DMSO-*d*₆): δ 170.6–170.1, 169.6–169.4, 151.2–150.7, 147.9–147.1, 100.9–99.3, 91.4, 73.5, 70.0–69.7, 68.1–67.9, 63.8–63.5, 54.7–54.0, 42.9–42.3, 22.8–22.7.

The MS (FAB) analysis in positive ion mode, was carried out on a VG ZAB SE instrument. Samples in thioglycerol (3-sulfanylpropane-1,2-diol) were loaded onto the stainless-steel target, which was bombarded with xenon atoms with a kinetic energy of 8 keV. Ions were accelerated using a potential of 10 kV. The analysis was carried out at BioCarb, Sweden. Ether **3**: MS (FAB): 3083 (*M* + *H*), 2957, 2830, 2702, 2578, 1550, 1424.

Computing. An in-house developed software *SynDe* (*Synthesis Design*) written in Zortech C/C++ V3.0 was used for creating the experimental design, for plotting the cumulative normal probability graphs, and for simulating and plotting the response surfaces. The UNSCRAMBLER program,¹² version 5.00 was used to estimate the regression coefficients, through the partial least-squares regression (PLSR) method. All computations were performed under DOS 5.00 on a COMPAQ DP 50M-340W, a 50 MHz 486DX2 microprocessor-based microcomputer.

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