

# Preparation of 6,9,12-Trioxatridecylmethylsilyl Substituted Silica, a New Stationary Phase for Liquid Chromatography

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The preparation of a substituted silica containing chemically inert ether groups is described, together with the synthesis of the derivative used for the substitution. The material shows excellent wetting properties in water containing solvent mixtures, and the utility as stationary phase for the chromatographic purifying of a partly protected decapeptide is shown.

The application of covalently bonded organic stationary phases is of great importance in modern liquid chromatography. Octadecylsilica (ODS-silica) is the most commonly used, but the range of substituted silica products is expanding because of the need for a larger variety in the selectivity of the materials. Also wetting properties and optimal mobile phase selection are of importance. In preparative liquid chromatography the solubility of the solute in the mobile phase is of great importance. In this case the variable parameter will be the stationary phase.

Thus it is valuable to have a wider range of stationary phases. We report the synthesis of a new stationary phase, a trioxatridecylsilica, which we think will be of great utility, due to its wetting properties.

The synthesis is described through all steps including the bonding procedure, and an example is given showing chromatography on the trioxatridecylsilica and for comparison on ODS-silica.

The trioxatridecylsilica has been shown to work by the reversed phase principle. However, it has properties very different from the ODS-silica, which makes it ideal for operation with mobile phases containing high concentrations of water.<sup>1</sup>

## ABBREVIATIONS

Pyr, Pyridine; -Si<sub>n</sub>, Silicon as part in a polymer matrix; DMF, *N,N*-dimethylformamide; TEAF, Triethylammonium formate; THF, Tetrahydrofuran.

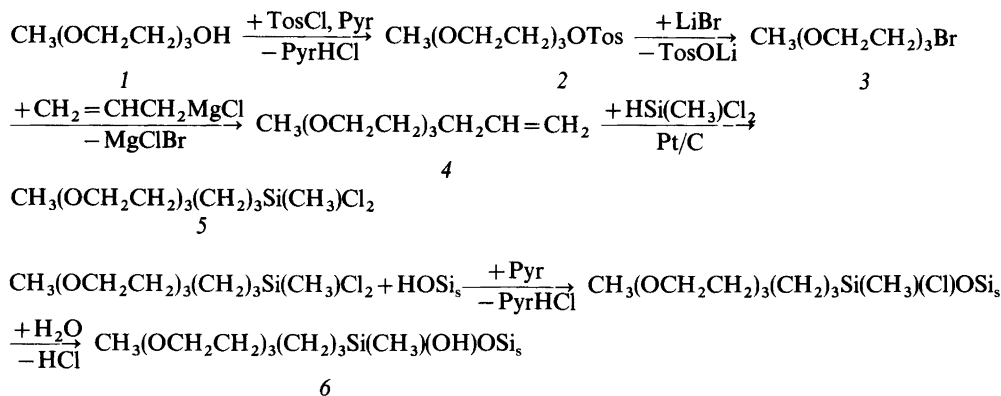
## RESULTS AND DISCUSSION

Most stationary phases are obtained by reacting pretreated silica with a chloro- or alkoxy silane containing the desired organic substituent.<sup>2</sup> To synthesize an appropriate chlorosilane two methods are usually employed. The first is a Grignard reaction between an alkyl- or arylmagnesium halide and usually either tetrachlorosilane, methyltrichlorosilane or dimethyldichlorosilane.<sup>3</sup> The second method involves the catalyzed addition between a silane containing one silicon-bound hydrogen and a (substituted) alkene.<sup>4</sup>

The substituted silica (6) was obtained by the reactions given in Scheme 1.

The sequence of reactions used to synthesize the 6,9,12-trioxatridecylmethylchlorosilane (5) was chosen because it proved impossible to make a Grignard compound of the bromide (3) due to elimination of ethene. The use of allylmagnesium chloride proved to be very convenient. No reaction to 1,5-hexadiene takes place due to the low solubility of the chloride in ether,<sup>5</sup> but it is very reactive. The addition to yield (5) proceeded in a high yield, an aspect which makes the addition approach to organic silanes very attractive as the yields in the Grignard method in our experience are generally mediocre.

Contradicting evidence exists as to which type of silane to use in the bonding reaction with silica.



Scheme 1.

Roumeliotis and Unger<sup>6</sup> prefer monochlorodimethylalkylsilanes due to the simple reaction mechanism involved and the fact that no silanol groups are formed by hydrolysis of unreacted chlorine atoms. Hemetsberger *et al.*<sup>7</sup> postulate a bidentate reaction and consequently favour dichloromonomethylalkylsilanes. Karch *et al.*<sup>8</sup> find no chromatographic difference between products obtained by reaction with either di- or trichlorosilanes. We decided to synthesize the dichloromonomethylsilane derivative, as we found that substantial evidence supported the theory of a bidentate reaction. However, we feel that this problem has not been solved, and we are going to synthesize the "ether" silane as the monochloroderivative in order to make a comparison.

The bonding reaction is rather difficult to evaluate due to the heterogenous process. Usually the products are characterized by either the carbon percent or the "coverage" in mmol/g of final product, or  $\mu\text{mol}/\text{m}^2$  of original silica. The carbon percent is obviously of limited value, when comparison is to be made between different reports. Theoretically the coverage in  $\mu\text{mol}/\text{m}^2$  should be the most informative, but care should be taken in the interpretation. The value is strongly dependent on the nature of the "parent" silica as well as the size of the substituent. However, the values obtained seem to be reasonable. The trioxatridecyl-substituent is slightly smaller than ODS, and a coverage a little higher than that of ODS on the same material is demonstrated ( $3.39 \mu\text{mol}/\text{m}^2$  for trioxatridecylsilica, and  $3.04 \mu\text{mol}/\text{m}^2$  for ODS-silica).

The chromatographic evaluation of the trioxatridecylsilica shows some interesting features (for details see Ref. 1). The product is completely wetted by water in contrast to ODS-silica. This is reflected in the reduced content of organic modifier in the mobile phase necessary to elute the peptide mixture as compared to that of ODS-silica. Also efficiency and selectivity were in this case better for the trioxatridecylsilica, allowing the use of a simple step gradient to separate the complicated mixture (Figs. 1 and 2).

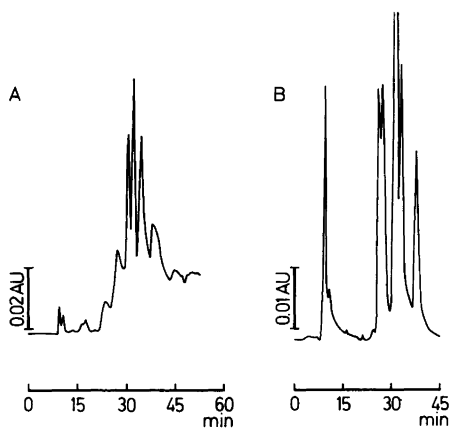


Fig. 1. Decapeptide, 10 mg. A: On ODS-silica; step gradient from 50% MeOH to 90% MeOH in 10% steps containing 10 mM TEAF, pH 6.2. B: On trioxatridecylsilica; twostep gradient: 60% MeOH for 10 min, then 10 mM TEAF in 40% MeOH, pH 6.2. Detected by UV absorption at 280 nm.

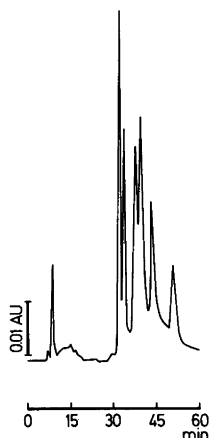


Fig. 2. Decapeptide, 10 mg, on trioxatridecylsilica; step gradient: 20 % THF for 10 min, 10 mM TEAF in 10 % THF, pH 6.2 for 10 min terminated by 10 mM TEAF in 15 % THF, pH 6.2. Detected by UV-absorption at 280 nm.

We feel that this bonded phase will be of great value as a completely water wettable reversed phase alternative to ODS-silica.

## EXPERIMENTAL

### Materials

Triethylene glycol monomethyl ether (techn.) from Fluka AG, Buchs, Switzerland, was distilled before use. Methylchlorosilane, platinum on charcoal and LiChrosorb Si-100, 10  $\mu$ m, were from Merck, Darmstadt.

### Synthesis of trioxatridecylmethylchlorosilane

*Triethylene glycol monomethyl ether p-toluenesulfonate* (2). A solution of *p*-toluenesulfonylchloride (4.5 mol, 857 g) in dichloromethane (1.25 l) was added with cooling to a mixture of triethylene glycol monomethyl ether (1) (3 mol, 492 g, 470 ml) and pyridine (4.5 mol, 355 g, 351 ml), ensuring that the temperature never exceeded 50 °C. After standing overnight at room temperature with stirring, water (1 l) was added and the pH of the suspension brought to 4 by addition of 6 M aqueous sodium hydroxide. After washing with water pH was brought to 1.5 by addition of 6 M hydrochloric acid. After

washing with water the organic phase was dried and evaporated. Yield of crude product: 744 g (78 %). This product was used in the next step without purification or characterization.

*3,6,9-Trioxadecyl bromide* (3). Lithium bromide (3 mol, 261 g) was added under slight cooling to a mixture of triethylene glycol monomethyl ether *p*-toluenesulfonate (2) (2 mol, 636 g) and DMF (640 ml) ensuring that the temperature never exceeded 50 °C. After standing overnight at room temperature with vigorous stirring, the mixture was diluted with ethyl acetate (2 l). After stirring for one h followed by filtration the solution was washed with water, dried and evaporated.

By distillation of the residue 418 g (92 %) of the product (3) was obtained, b.p. 57–61 °C at 0.05 mmHg.  $^1\text{H NMR}$  (270 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.39 (3H, s, methyl protons), 3.82 and 3.47 (2H, t and 2H, t, methylene protons between oxygen and bromine).

*6,9,12-Trioxatrideca-1-ene* (4). By addition of allyl chloride (1 mol, 76.5 g, 81.5 ml) in ether (150 ml) to magnesium turnings (1.1 mol, 27 g) in ether (150 ml) during 16 h and with ice cooling, a suspension of allylmagnesium chloride was made. By addition of dry tetrahydrofuran the allylmagnesium chloride dissolved and the excess of magnesium was removed by decantation. During 30 min 3,6,9-trioxadecyl bromide (3) (0.5 mol, 113 g, 89 ml) was added to the refluxing solution of the Grignard reagent. After stirring for further 15 min water (250 ml) was slowly added, and pH adjusted to 1 using 6 M hydrochloric acid. After washing with water, drying and evaporating the product was distilled. Yield: 76 g (81 %), b.p. 49–50 °C, 0.2 mmHg.  $^1\text{H NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.30 (3H, s, methyl protons), 2.04, 4.95 and 5.78 (5H, complicated system from allyl group), after decoupling <212 Hz:  $\delta$  4.95 and 5.78 (3H, dq and dd, showing characteristic pattern of an ABX-system).

*6,9,12-Trioxatridecylmethylchlorosilane* (5). 6,9,12-Trioxatrideca-1-ene (4) (0.4 mol, 75 g, 82 ml) and 10 % Pt/C catalyst (0.2 g) were placed in a flask immersed into an oil bath. While keeping the temperature just below 95 °C methylchlorosilane (0.6 mol, 70 g, 60 ml) was added to the mixture. After the addition the mixture was stirred overnight at 80 °C, followed by distillation of the product. Yield: 106 g (85 %), b.p. 98–102 °C, 0.05 mmHg.  $^1\text{H NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.29 (3H, s, oxygen-bound methyl protons), 0.63 (3H, s, silicon-bound methyl protons), 1.00 (2H, complicated system from silicon-bound methylene protons), 1.39 (6H, complicated system from carbon-bound methylene protons).

### Preparation of the trioxatridecylsilica

LiChrosorb Si-100, 10  $\mu\text{m}$  (125 g) was dried overnight at 150 °C and 0.01 mmHg in the reaction flask. After drying, dry nitrogen was leaked into the flask, and the material was mixed with dry toluene (500 ml), 6,9,12-trioxatridecylmethylchlorosilane (0.27 mol, 82 g, 78 ml), pyridine (0.8 mol, 63 g, 64 ml) and the suspension stirred mechanically under reflux overnight. After cooling, the slurry was transferred to a closed glass column fitted with a glass filter disc in the bottom, and washed with dry toluene (800 ml), acetone (800 ml), methanol (800 ml), 1:1 methanol–water V/V (800 ml) and acetone (800 ml).

After drying at 110 °C and 0.01 mmHg overnight the procedure described above was repeated, using dry toluene (500 ml), trimethylchlorosilane (0.27 mol, 29.3 g, 34 ml) and pyridine (0.40 mol, 31 g, 32 ml). Coverage: 3.39  $\mu\text{mol}/\text{m}^2$ .

### Chromatographic procedure

The preparative system was based on a Jobin-Yvon column (4 cm diameter, maximum length 50 cm), modified with a sixport injection valve (Whitey) with a teflon loop of 1 ml. The solvent delivery was effected by a Lewa FD pump connected to a pressure transducer for pressure recording and a Fluidyne flowmeter for flow control. A Uvicord S set for detection at 280 nm was used as detector. The amount of stationary phase (either trioxatridecylsilica or ODS-silica) packed into the column was 135–150 g. The detailed chromatographic conditions are given in the figure legends. As test substance, a crude product from a peptide synthesis was used. The structure of the peptide was: Glu-Arg( $\text{NO}_2$ )-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Thr, the C-terminal decapeptide of the B-chain of human insulin.

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