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Preparation, characterization and application of a stationary chromatographic phase from a new (+)-tartaric acid derivative

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ABSTRACT

The preparation, characterization and application of a new stationary phase derived from 1,4-cyclohexanedione and diethyl (+)-tartrate are described. A suitable TADDOL for immobilization has been synthesized and grafted to a γ-mercaptopropylsilylated silica gel. The resulting modified stationary phase has been characterized and its ability to separate enantiomers has been studied. While the free TADDOL in solution was able to resolve a range of enantiomers, the resolving properties were lost on immobilization. Solid state ^{13}C CPMAS NMR of the new stationary phase was used to explain the lack of stereoselective recognition.

Chiral stationary phases (CSPs) are of fundamental importance in liquid chromatography for the resolution of racemates into their pure enantiomers.[1] Partial resolution of racemic camphor with a lactose-based stationary phase was initially demonstrated by Henderson and Rule as early as 1938.[2] Since then, numerous examples of chiral stationary phases have been reported in the literature.[3–5]

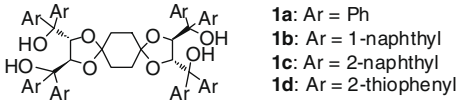
TADDOLs, (+)-tartaric acid derivatives, have been shown to be useful as host molecules for the resolution of racemic mixtures.[6,7] Tartaric acid-based solid phases have been shown to be promising chiral selectors for enantioselective liquid chromatography.[8–13] Recently, we prepared a new generation of TADDOLs **1a–d** derived from 1,4-cyclohexanedione and diethyl (+)-tartrate (Fig. 1).[14]

By using a series of ^1H NMR studies, we demonstrated the ability of these new TADDOLs to interact stereoselectively with the guest alcohols menthol and glycidol.[14] Only a few other studies of this new TADDOL family have been reported so far.[15,16] Based on our promising results and the observed enantioselectivities in solution, we decided to study the possibility of using this new generation of TADDOLs as chiral selectors for liquid chromatography.

Herein, we report the synthesis of a new vinyl-containing TADDOL **4**, which is suitable for immobilization on a solid phase. The TADDOL **4** was further coupled to a silica gel which was characterized by a series of analytical techniques. Finally, its performance as a stationary phase was evaluated by chromatography using a range of non-chiral and chiral analytes.

A series of ^1H NMR titration experiments was previously performed in our laboratory.[14] These were performed in $CDCl_3$ and it was possible to determine apparent dissociation constants (app. K_d) (Table 1) for the complex formation between **1a** and the pure enantiomers of menthol and glycidol (Fig. 2). The mode of interaction was mainly due to hydrogen bond formation between the hydroxy groups of TADDOL **1a** and the alcohol guests.

Importantly, we demonstrated the ability of the phenyl-containing TADDOL **1a** to recognize, with enantioselectivity, both menthol and glycidol. The difference in recognition between the enantiomers was not general throughout the study and the TADD-



1a: Ar = Ph
1b: Ar = 1-naphthyl
1c: Ar = 2-naphthyl
1d: Ar = 2-thiophenyl

Figure 1. Structures of TADDOLs **1a–d**.

Table 1
Dissociation constants [K_d (μM)][a] for complex formation between the phenyl TADDOL **1a** and guests (chiral alcohols)

Entry	Guest	K_d[a] (μM)
1	(−)-Menthol	550 ± 30
2	(+)-Menthol	100 ± 30
3	(−)-Glycidol	190 ± 60
4	(+)-Glycidol	630 ± 20

[a] Apparent dissociation constants were calculated with non-linear line fitting to a one-site model with the software package Prism (version 3.03, GraphPad Software, USA).

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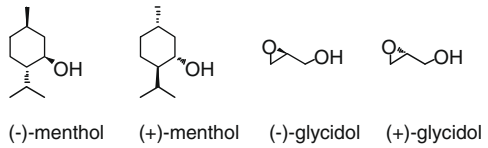


Figure 2. Structures of the tested chiral alcohols.

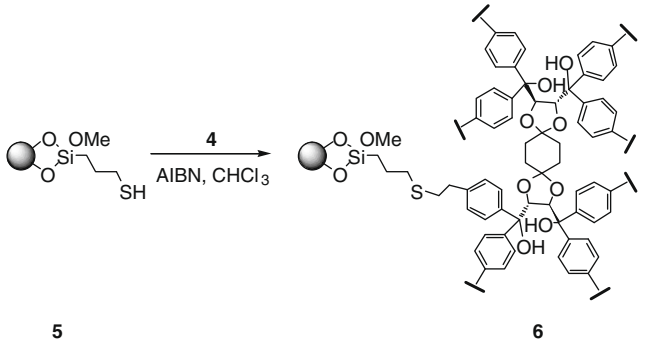
OLs **1b** and **1d** did not discriminate at all.[14] The observed enantioselectivities and differences in dissociation constants (Table 1) between enantiomers encouraged us to use this new generation of TADDOLs as chiral selectors for liquid chromatography, especially for the resolution of small chiral alcohols.

The design of a suitable TADDOL for immobilization on a solid support was based on the work described by Degni et al.,[17] where a vinyl-containing TADDOL was immobilized on silica gel. In addition to an anchoring group, a suitable TADDOL should possess a similar chemical structure to **1a**, with sufficiently bulky pendant side chains to generate a chiral 'cavity' to host the target analytes. The critical effect of the steric bulk in enantioselective recognition of TADDOLs has previously been highlighted.[14] Therefore, we prepared the vinyl derivative TADDOL **4** in two steps from the 1,4-cyclohexanedione (**2**) and (2*R*,3*R*)-(+)-tartaric ethyl ester (Scheme 1). Reaction between the tetraester **3** and the Grignard reagent derived from 4-bromostyrene afforded the target molecule (50% isolated yield after purification).

The TADDOL **4** was attached to the γ-mercaptopropylsilylated silica **5**[18] under radical conditions (AIBN) to afford the silica-bound derivative **6** (Scheme 2).

The silica gels **5** and **6** were analyzed by Raman spectroscopy, which is a more sensitive method for silica-bound derivatives than FT-IR spectroscopy. The Raman spectrum of the γ-mercaptopropylsilylated silica **5** exhibited a very strong band at 2581 cm^{-1} due to the S–H bond. This band was found to be very weak in the Raman spectrum of **6** which clearly indicated that the S–H moieties of **5** had reacted with the double bonds of TADDOL **4**, or potentially with each other to form disulfide bonds.[19] Additionally, bands corresponding to different vibration modes of the immobilized TADDOL were observed at ca. 3060–3007 (CH arom) on the Raman spectra of **6**. Other characterization of the silica gels **5** and **6** included elemental analysis, nitrogen adsorption isotherm measurements and average pore diameter determinations (Table 2).

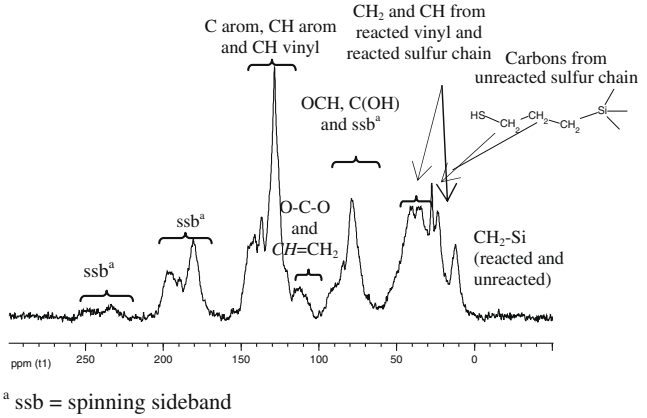
The ^{13}C NMR spectrum of the TADDOL **4** was partially resolved by using a DEPT experiment ($\theta = 135°$). It was noticed that the chemical shifts at 114.2, 114.0 and 113.8 ppm correspond exclusively to the $H_2C{=}CH$ moieties present in **4**. Solid state NMR (^{13}C CPMAS NMR) of the derivatized silica gel **6** was also used as a characterization method. The ^{13}C CPMAS NMR spectrum of the solid phase **6** is depicted in Figure 3. The region at 30–50 ppm, which corresponded to the CH_2 groups from the cyclohexane ring, displayed significantly



Scheme 2. Preparation of the chiral solid phase **6**.

Table 2
Elemental analysis, nitrogen adsorption isotherm measurements and average pore diameter evaluation values for the silica gels **5** and **6**

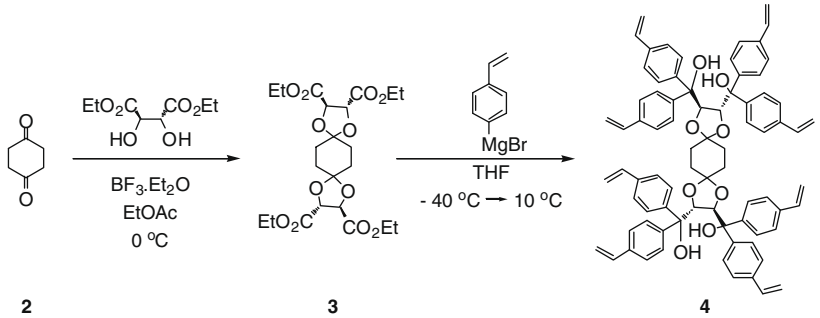
Entry	Experiment	5	6
1	% C found	10.25	31.70
2	% H found	2.30	3.50
3	% O found	2.00	4.70
4	% S found	6.75	4.35
5	BET surface area ($m^2\ g^{-1}$)	487.8129	866.7478
6	Average pore diameter (Å)	67.1932	91.4067



[a] ssb = spinning sideband

Figure 3. ^{13}C CPMAS NMR spectrum and assignments of the derivatized silica gel **6**.

greater intensity than the region at 112–115 ppm (vinyl $H_2C{=}CH$). This observation indicated that the vast majority of the vinyl groups present in the TADDOL **4** had reacted during the polymerization process. The solid state NMR spectrum of **6** also indicated that most of the SH groups had reacted, since the peak at 27 ppm, due to the S–



Scheme 1. Preparation of the vinyl-containing TADDOL **4**.

CH_2–CH_2 groups, was found to be much smaller than the Si–CH_2 peak at 12 ppm. This result supported our previous interpretation based upon Raman spectroscopy analysis. Collectively, these observations allowed us to conclude that the TADDOL **4** had been immobilized on the silica gel **5**.

Due to incompatibility issues with reversed-phase LC–MS and UV detection, it was not possible to use chloroform as the mobile phase for the liquid chromatographic experiments. In terms of dielectric constants (DEs), hydrogen bond donor parameters (HBDPs) and hydrogen bond acceptor parameters (HBAPs), it was concluded that the binary solvent mixture, 2-propanol (IPA, 10% in volume) in *n*-hexane should display properties sufficiently similar to chloroform.[20] Therefore, we decided to evaluate the new TADDOL-grafted solid phase in the binary solvent 2-propanol/*n*-hexane. However, the TADDOL **4** was not soluble in this mixture and comparative NMR-titrations could not be performed in this solvent mixture.

In an initial investigation of the binding properties of the modified solid phase, retention factors (k') were obtained for toluene, 2-phenylphenol and 2-methoxyphenol in a range of different mixtures of *n*-hexane and IPA (Table 3). A very weak binding was observed for toluene (Table 3, entry 1). This result was probably the consequence of very weak π–π interactions between the solid phase **6** and toluene. On the other hand, 2-phenylphenol and 2-methoxyphenol displayed significantly longer retention times than toluene. Their retention times could be further increased by lowering the polarity of the mobile phase. This behaviour supported the earlier studies on these TADDOLs where the enantioselective interactions were mainly believed to be electrostatic in nature for this type of analytes. The weakly acidic phenyl hydroxy group in both the tested phenols readily formed hydrogen bonds with the hydroxy groups present in **6**, resulting in higher retention factors (Table 3, entries 2 and 3) in a less polar solvent. These initial results further indicated that the recognition cavities of the TADDOL **6** were available for binding after immobilization. A reference column containing only non-derivatized silica gel **5** with free thiol groups displayed significantly lower retention factors in control experiments in apolar solvents.

Based on these preliminary binding experiments, which illustrated strong electrostatic interactions between the stationary phase **6** and the various non-chiral compounds, we decided to study the chiral recognition ability of the TADDOL-grafted silica gel **6**. The changes in retention factors (Table 4, entries 1–4) clearly indicated that the chiral analytes interacted with the derivatized silica gel. Unfortunately, no enantiomeric recognition of either menthol or glycidol by the grafted silica gel **6** occurred.

While the solution behaviour of the free TADDOLs, previously shown to display enantioselectivity,[14] greatly differed from anchored **6**, it was still surprising to observe that the resolving capacity vanished upon immobilization. The LC experiments showed, beyond doubt, that the analytes had free access to the hydroxy groups inside the solid support **6** indicating that the loss of selectivity was not caused by poor access. This was further supported by the large pore size of the derivatized silica (Table 2). A more likely explanation was instead loss of rotational freedom of the pendant phenyl groups. These were no longer free to rotate in the solid state. It may be that the nearly complete cross-linking in **6**, as shown by both solid state NMR and Raman spectroscopy, disrupted the chiral surroundings around the hydroxy groups. In solution, these groups were essential for enantioselectivity[14] and it is very likely that they were shifted from their stable chiral equilibrium state during the immobilization process. The free radical polymerization is a strongly exothermic and reactive process and it generated a highly rigid stationary phase in this case. We could therefore not rule out radical disulfide formation which would further add to the rigidification of the system.

A solution to this challenge would be to design a mixed TADDOL in which only one or two anchoring groups are attached to the structure. That would yield a stationary phase with recognition elements that have more freedom to adopt the correct conformation.

In conclusion, a new (+)-tartaric acid derivative has been synthesized and immobilized on silica gel to yield a novel TADDOL-grafted solid phase. This new derivatized silica gel has been characterized using various chemical, physical and spectroscopic methods. Interactions between the solid phase and several analytes have been demonstrated. Although no chiral separation was observed, the loss of the ability of the TADDOL to engage in enantioselective recognition could be explained by the loss of freedom of the system. Alternative coupling strategies and more suitable TADDOL derivatives are suggested improvements for further development of these stationary phases.

Table 3
Retention factors found for toluene, 2-phenylphenol and 2-methoxyphenol in various mobile phases[a]

Entry	Analyte	(k')		
1	Toluene	0.09[b]	0.09[c]	0.11[d]
2	2-Phenylphenol	0.32[b]	0.64[c]	1.70[d]
3	2-Methoxyphenol	0.44[b]	0.89[c]	2.17[d]

[a] Flow: 0.8 ml/min; injection volume: 20 μl; detection: UV 254 nm.
[b] Mobile phase: *n*-hexane/IPA: 60/40.
[c] Mobile phase: *n*-hexane/IPA: 80/20.
[d] Mobile phase: *n*-hexane/IPA: 95/5.

Table 4
Retention factors found for (−)-menthol, (+)-menthol, (−)-glycidol and (+)-glycidol[a]

Entry	Mobile phase	Analyte	(k')	Selectivity factor (α)
1	*n*-Hexane/IPA: 95/5	(−)-Menthol	1.08	n.s.[b]
2	*n*-Hexane/IPA: 95/5	(+)-Menthol	1.08	n.s.[b]
3	*n*-Hexane/IPA: 99/1	(−)-Menthol	2.62	n.s.[b]
4	*n*-Hexane/IPA: 99/1	(+)-Menthol	2.62	n.s.[b]
5	*n*-Hexane/IPA: 90/10	(−)-Glycidol	2.48	n.s.[b]
6	*n*-Hexane/IPA: 90/10	(+)-Glycidol	2.48	n.s.[b]

[a] Flow: 0.8 ml/min; injection volume: 20 μl; detection: UV 254 nm.
[b] n.s. = no separation was observed under these experimental conditions.

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Supplementary data

Supplementary data (synthesis and characterization of the TADDOL **4**, preparation of the silica gels **5** and **6**, chromatographic and solid state NMR experimental procedures) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.02.101.

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