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Tetrahedron Letters
Tetrahedron Letters 47 (2006) 8721–8726

Highly efficient immobilization of *Cinchona* alkaloid derivatives to silica gel via *click chemistry*

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Received 6 September 2006; revised 2 October 2006; accepted 4 October 2006

Abstract—*Click chemistry* was adapted to the immobilization of various *Cinchona* alkaloid derivatives bearing alkyne functionality onto azide-modified silica gel surfaces. The developed protocol employs very mild reaction conditions, with catalytic amounts of copper(I) iodide (1–5 mol %) in acetonitrile at room temperature, ensuring complete chemical integrity of the multi-functional ligands. The utility of this approach is demonstrated by the attachment of didehydroquinine *tert*-butylcarbamate to azido-grafted silica gel to produce an effective chiral stationary phase for HPLC enantiomer separation. A comparison of the chromatographic behavior of this *click-immobilized* phase with that of a commercially available thioether-linked chiral stationary phase revealed very similar performance characteristics for various model analytes. This observation suggests that the *click-immobilization* may offer an appealing alternative to the established silica gel-grafting technologies, allowing for the specific benefits of high and readily controllable loading levels under noninvasive conditions.

1. Introduction

Immobilization of highly functionalized (bio)molecules onto the surfaces of solid supports is a frequent requirement in the development of reusable catalysts,[1] chromatographic materials,[2] sensors,[3] chip-type analytical devices,[4] and solid-phase synthesis strategies.[5] Most importantly, dedicated linking procedures must preserve the chemical integrity and the functional activity of the attached molecular systems, utilizing mild and noninvasive immobilization chemistries. In addition, immobilization protocols should address a broad range of targets and allow for convenient control of ligand orientation and surface density.[6]

The recent discovery of the Cu(I)-catalyzed 1,3-dipolar cycloaddition of azides to alkynes has provided the most powerful *click chemistry* tool for conjugation between appropriately functionalized binding partners via an 1,2,3-triazole linkage.[7] The underlying cycloaddition reaction fulfils many of the crucial requirements for mild surface immobilization. These include modularity, favorable thermodynamics and therefore excellent coupling yields, ease of introduction of the required azide and alkyne groups both of which are compatible with a broad repertoire of functionality and reaction conditions. These appealing characteristics of the azide-alkyne *click chemistry* (AACC) reactions has led to numerous applications in various fields of organic, medicinal, polymer, and material chemistry including biochemistry and molecular biology.[8] The great potential of AACC for the covalent attachment of ligands to chromatographic supports, however, has been recognized only recently. Pioneering work in this direction was disclosed by the groups of Finn and Frechet, communicating AACC protocols for the immobilization of affinity ligands onto biocompatible agarose[9] and polyacrylamide-type beads.[10] To the best of our knowledge, AACC-based technology for grafting small-molecule receptors onto silica gel, the most prominent chromatographic matrix to date, has not been reported so far.[11]

Our ongoing research projects in the fields of *Cinchona* alkaloid-based chiral stationary phases (CSPs),[12] sensors,[13] switches,[14] and organocatalysts[15] challenged us to devise mild and generally applicable approaches to the covalent immobilization of this 'privileged' class of ligands[16] onto silica gel surfaces. Currently, relatively few practical, noninvasive methods are available for the covalent attachment of *Cinchona* alkaloid scaffolds onto silica supports.[15,17] The majority of these

Keywords: *Click chemistry*; Immobilization; Chiral stationary phase (CSP); *Cinchona* alkaloids; Enantioseparation.
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0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.10.018

approaches exploit the free radical addition of thiol-modified silica gels to the vinyl group at the quinuclidine moiety to produce a thioether-linkage offering the benefits of chemical inertness and stability. In general, *Cinchona* alkaloid-derived ligands attached to silica via this site retain their catalytic or molecular recognition capacities.[12] This convenient strategy, however, may prove incompatible with *Cinchona* scaffolds sensitive to free radicals and/or ligands bearing radical capturing functionality.[18] In addition, to proceed in a high yield, the free radical addition protocol requires thermal or photochemical activation over extended time periods, which further restricts the range of addressable ligands to *Cinchona* substrates compatible with these conditions. Facing these limitations, we envisioned that Cu(I)-triggered AACC may offer a milder and more broadly substrate-compatible immobilization strategy for *Cinchona* alkaloid derivatives. We took additional encouragement from the fact that azido[19] or acetylene groups[20] required for AACC immobilization can be easily introduced into *Cinchona* alkaloids following well-established synthesis protocols.

In this contribution, we present the preliminary results concerning the preparative aspects of AACC immobilization of various *Cinchona* alkaloids alkyne-derivatives onto azido-modified silica gels. The impact of substrate nature and reaction conditions on the efficiency and kinetics of AACC immobilization are discussed in detail. The practical applicability of the developed AACC immobilization protocol is demonstrated by the successful ambient temperature grafting of 10,11-didehydroquinine *tert*-butylcarbamate onto azidopropyl-modified silica gel. The chromatographic chiral recognition characteristics of the resultant triazole-linked CSP for a series of acidic model analytes are shown to be in good agreement to those of a thioether-linked reference CSP with comparable selector loading level.

2. Discussion

Azido-modified silica gels were prepared in a two-step procedure, involving surface pre-activation with haloalkylsilanes followed by a nucleophilic halide/azide exchange. Different types of base silica materials were tested. As a candidate carrier material for solid-phase supported reagents and catalysts, we evaluated relatively inexpensive flash-type silica gel (40–65 μm irregular particles, 6 nm pore diameter, 500 m^2/g specific surface area). For the development of chromatographic materials, however, we employed HPLC grade silica gel (5 μm spherical particles, 12 nm pore diameter, 330 m^2/g specific surface area). Reacting flash-type silica gel under thermal conditions with chloropropyltrimethoxysilane produced a material with a loading level of 700 μmol chloropropyl groups/g. With the HPLC grade silica, these reaction conditions led to a higher loading (980 μmol/g). Attempts to immobilize 11-bromoundecyltrimethoxysilane under analogous conditions onto flash-type silica resulted in a diminished ligand loading (365 μmol/g), most probably due to the enhanced steric requirements associated with the longer alkyl chains.

The pre-activated materials were transformed into the corresponding azido-modified silica gels using an excess of NaN_3 in DMSO with conventional or microwave-assisted heating. Both sets of conditions were found to promote an efficient halide/azide exchange. For the HPLC grade material, thermal conditions produced the corresponding azidopropyl-modified silica gel (AzPrS) with a conversion of 90% (azide loading 870 μmol/g). A comparably favorable level of loading (87% conversion, azide loading 760 μmol/g) could be obtained when microwave heating was employed. Even higher levels of conversion (95% conversion, corresponding to an azide loading of 660 μmol/g) were observed for the pre-activated materials prepared with flash-type silica. Essentially quantitative halide/azide exchange was achieved with bromoundecyl flash silica under microwave conditions, furnishing 11-azidoundecyl silica (AzUS) with an azide loading of 365 μmol/g.[21]

With the azido-modified silica supports in hand, a set of alkyne-functionalized *Cinchona* alkaloid derivatives (**1**–**4**, Fig. 1) were selected for AACC studies.[22] As a key intermediate, 10,11-didehydroquinine **1** was prepared via a bromination/dehydrohalogenation sequence, employing a modified version of the procedure reported by Hoffmann.[20] The corresponding *tert*-butyl and 3,5-dinitrobenzoylcarbamate derivatives of 10,11-didehydroquinine **2** and **3** were obtained by reacting the parent compound with the corresponding isocyanates in refluxing toluene.[12b] Carbamate **2** represents an alkyne analog of *tert*-butylcarbamoylquinine, a well-established commercial chiral selector developed by our group.[12,23] To probe the sensitivity of the AACC immobilization concept toward steric factors, 9-*O*-propargylcinchonidine **4** was included into the set of *Cinchona* alkaloid-derived substrates. In this case, the alkyne functionality is

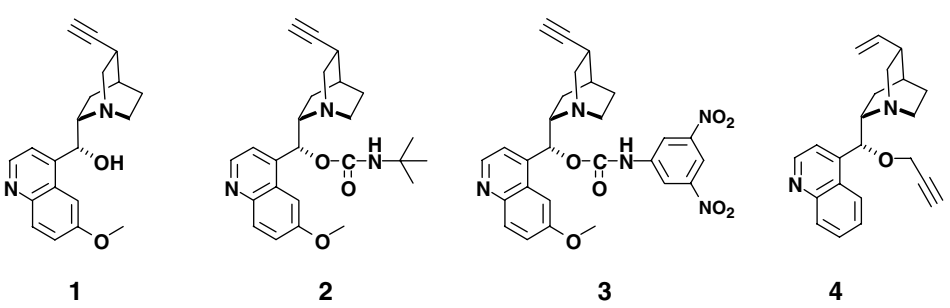
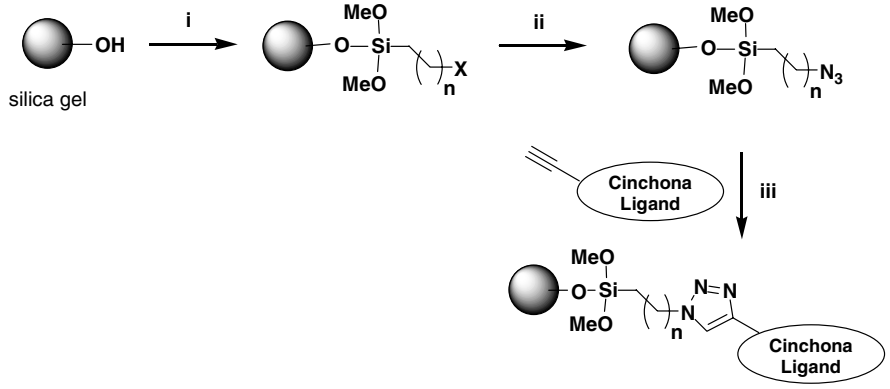


Figure 1. Structures of alkyne-modified *Cinchona* alkaloids.

attached to the sterically rather congested 9-*C*-stereogenic center (Scheme 1).

To establish an AACC-mediated immobilization performing at an ambient temperature, various sets of reaction conditions and catalyst systems were systematically screened. First we attempted to promote immobilization by shaking the suspensions of the azido-modified silica gels with the corresponding *Cinchona* alkyne derivatives (0.9 equiv) in the presence of various Cu(I)-catalysts in different solvents. Specifically, two different established protocols were tested, using either equimolar amounts CuI in presence of an excess of a tertiary amine in polar organic solvents (Meldal protocol,[7b]) or, alternatively, a catalytic amount of $CuSO_4$/ascorbate in hydroorganic media (Sharpless protocol.[7c]) Different levels of ligand immobilization were achieved for *Cinchona* derivatives **1** and **4**, with the Meldal protocol showing superior performance (88% versus 27% conversion, entries 2 and 1 in Table 1). We reasoned that the poor grafting efficiency observed with the Sharpless protocol may reflect the consequences of an insufficient surface wetting by the hydroorganic reaction mixture. Evidently, an efficient AACC immobilization requires well-wetting low-viscosity organic media such as acetonitrile, which provides the additional benefit of accelerated intraparticle diffusion and thus high ligand and catalyst concentrations at the azido group-grafted surface. From the practical viewpoint, however, the high CuI concentrations routinely employed in Meldal-type AACC procedures complicated the isolation of the modified silica gels, requiring numerous high-stringency washing steps to remove precipitated and surface-adsorbed copper salts. This drawback could be successfully addressed by diminishing the CuI loading from equimolar to truly catalytic concentration levels. After a considerable experimentation, a homogeneous solution of the *Cinchona* derived alkyne derivative (0.9 equiv) in degassed acetonitrile comprising 5 mol % CuI and DIPEA (3 equiv) was identified as a convenient and broadly applicable reaction system for AACC immobilization.[24] The incubation of a mechanically agitated suspension of AzPrS with this mixture comprising ligand **1** at 25 °C for 48 h produced a material showing a grafting level of 750 μmol/g, corresponding to 86% conversion of the initially available azido groups. This represents a more than threefold enhancement in grafting efficiency as compared to the heterogeneous Sharpless protocol (entry 1, 27% conversion). Similarly favorable grafting yields were obtained for *Cinchona* derivatives **2** and **3** (Table 1, entries 5 and 7), for which conversion levels of 71% and 60% could be achieved, respectively. The



Scheme 1. (i) $n = 2$, X = Cl (3-chloropropyltrimethoxysilane) or $n = 10$; X = Br (11-bromodecyltrimethoxysilane), toluene, reflux, 18 h; (ii) DMSO, NaN_3, 48 h; (iii) alkyne-functionalized *Cinchona* ligands **1**–**4**, Cu(I)I 1–5 mol %, CH_3CN, rt, 48 h.

Table 1. Immobilization of *Cinchona* alkaloids derivatives to the azido-modified supports by *click chemistry*

Run[a]	Azido-silica gel (azide loading, mmol/g)	Cinchona-type ligand (equiv)	Catalyst (mol %)[b]	Method[c]	Achieved ligand loading (mmol/g)[d]	Conversion of the azido groups (%)
1	AzPrS (0.87)	1 (0.9)	$CuSO_4$ (1)	A	0.18	27
2	AzUS (0.36)	4 (0.9)	CuI (100)	B	0.32	88
3	AzPrS (0.87)	1 (0.9)	CuI (5)	B	0.75	86
4	AzPrS (0.87)	2 (0.9)	CuI (1)	B	0.55	63
5	AzPrS (0.87)	2 (0.9)	CuI (5)	B	0.62	71
6	AzPrS (0.87)	2 (0.3)	CuI (5)	B	0.30	34[e]
7	AzPrS (0.87)	3 (0.9)	CuI (5)	B	0.53	60
8	AzUS (0.36)	1 (0.9)	CuI (1)	B	0.34	93
9	AzUS (0.36)	2 (0.9)	CuI (1)	B	0.28	77
10	AzUS (0.36)	4 (0.9)	CuI (5)	B	0.29	79

[a] Data relate to the average value of duplicate runs.
[b] 1 M $CuSO_4$ aq and solid sodium ascorbate (10 mol %) or acetonitrile solution of CuI (~50 mg/5 mL) were used.
[c] Method A: THF/H_2O 2:1, 40 °C, 24 h, method B: DIPEA (3 equiv), ACN, rt, shaking, 50 h.
[d] Loading was calculated by elemental analysis from the gain of C and N contents.
[e] *Cinchona* ligand employed as a limiting reagent (100% conversion).

kinetics of the AACC grafting procedure was monitored for *Cinchona* derivative **2** as a representative ligand by HPLC, employing the corresponding 10,11-dihydro analog as an internal standard. The respective kinetic graph depicted in Figure 2 indicates that even at an ambient temperature the rate of ligand immobilization is relatively fast, with 50% of the available azido groups capturing substrate in less than 7 h. Saturation is essentially observed after 24 h, most probably due to the steric demands of the immobilized ligands rendering the remaining azido groups inaccessible for further reaction.

A remarkable finding is the highly efficient immobilization of *Cinchona* derivative **3** (60% conversion, entry 7) containing the 3,5-dinitrobenzoyl functionality, the presence of which is known to compromise the free radical addition reaction with thiol-modified silica gels.[18] This recommends the developed AACC protocol as an excellent alternative immobilization strategy for ligands being incompatible with the free radical chemistry.

Although copper(I) salts are tolerated by a broad range of functionality at an ambient temperature, their presence still may cause problems with substrates prone to transition metal-triggered transformations. To limit the risk of potential copper-induced substrate damage, the efficiency of the AACC immobilization protocol was assessed under the condition of limiting CuI concentrations. We were pleased to find that AACC-mediated ligand attachment proceeds efficiently even with catalyst loading as low as 1 mol % CuI. Thus, under these conditions ligand **2** was clicked onto AzPrS with 63% conversion (entry 4), a figure being close to the grafting level obtained with 5 mol % CuI (71% conversion, entry 5). The same was true for ligands **1** and **2** (entries 8 and 9), which were grafted onto AzUS with 93% and 77% conversions, respectively.

The developed AACC immobilization protocol allows us to create ligand-modified silica gels with high surface loadings, which compare well to or even exceed those achievable with the established free radical addition protocol to thiol-modified silica gels (490 μmol/g of selector **2** is the highest loading obtained in our laboratory). As an additional attractive feature, AACC provides the opportunity to create ligand-modified silica gels with predefined grafting levels. As demonstrated by entry 6, complete immobilization can be accomplished by offering *Cinchona* ligand **2** as a limiting reagent (0.3 equiv relative to surface-grafted azido groups). This mild and simple-to-control loading procedure may prove particularly valuable to study the impact of ligand density on the activity or molecular recognition capacity of surface-attached catalysts and receptors.

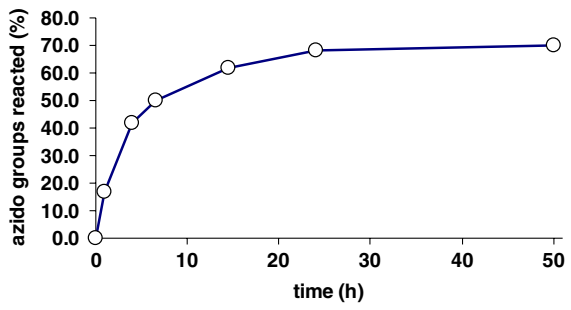


Figure 2. Kinetics of the click immobilization of Cinchona ligand **2** to AzPrS.

To demonstrate the capacity of the AACC to provide fully functional silica-grafted *Cinchona* receptors, a triazole-linked chiral stationary phase was prepared by attaching ligand **2** onto AzPrS and its chiral recognition profile compared with that of a structurally related, but thioether-linked CSP. To cancel out any deviations in the chromatographic behaviors arising from the variations in ligand accessibility and/or density CSPs with similar but rather diluted surface coverages (triazole-linked CSP: 230 μmol/g, thioether-linked CSP 202 μmol/g) were created, employing controlled loading

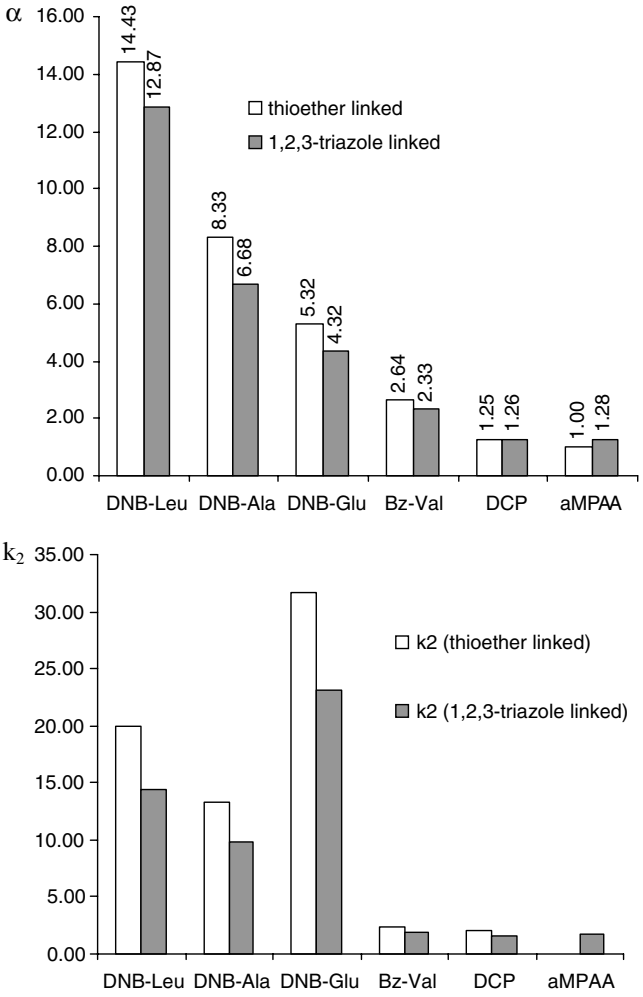


Figure 3. Comparison of enantioselectivity (α—upper panel) and retention behavior (k_2 values—lower panel) of the thioether- and triazole-linked phase quinine *tert*-butylcarbamate CSPs (DNB: 3,5-dinitrobenzoyl, Bz: benzoyl, DCP: 2-(2,4-dichlorophenoxy)propionic acid, aMPAA: α-metoxyphenylacetic acid). Columns (100 × 4 mm); mobile phase: MeOH:AcOH:NH_4OAc 98:2:0.5 (v/v/w); flow rate 1 mL/min; 25 °C; UV detection at 254 nm; injection volume 5 μL, concentration of analytes ~1 mg/mL. The void volumes were determined using acetone as a nonretained marker.

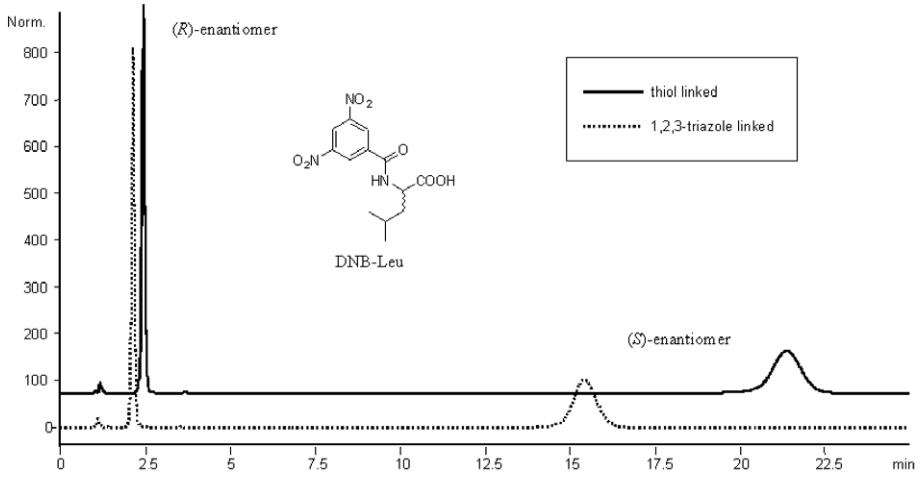


Figure 4. Enantioseparation of DNB-Leu on a triazole-linked column and thioether-linked Prontosil column (chromatographic conditions as in Fig. 3 above).

procedures. The columns packed with these materials were tested with a small set of chiral model analytes under polar organic mobile phase conditions. The observed chromatographic enantiomer separation data are summarized in Figure 3, and the representative chromatograms for 3,5-dinitrobenzoyl leucine are depicted in Figure 4. The enhanced conformational constraints of the triazole-linker appear to diminish analyte retention relative to the more flexible thioether congener. However, both CSPs exhibit identical enantiomer elution order and very similar levels of enantioselectivity, suggesting that the chiral recognition capacity of the immobilized *Cinchona*-type selectors are largely retained.

In conclusion, a robust AACC strategy for the immobilization of alkyne-functionalized *Cinchona* alkaloid derivatives onto azido-functionalized silica gel surfaces was developed. In contrast to most of the established immobilization procedures, the AACC protocol combines the benefits of operational simplicity, exceptionally mild grafting conditions, and allows for a convenient control over surface coverages. We are confident that the reported AACC immobilization strategy will find practical applications in fields beyond chromatographic material technology, such as catalyst and material research and life sciences. Applications along these lines are currently under evaluation in our laboratories and will be reported in due course.[25]

Acknowledgments

A Marie-Curie Fellowship awarded to K.K. (No. MEIF-CT-2004-011165) is kindly acknowledged. We thank Ms. Jadwiga Gajewy for the assistance in the experimental work.

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21. *3-Azidopropyl silica gel*: 10.0 g of 3-chloropropylsilica (0.98 mmol/g) was suspended in 60 mL 0.5 M NaN_3 in DMSO, 50 mg of tetrabutylammonium iodide was added and the mixture was mechanically stirred at 80 °C for 48 h. The silica gel was filtered and washed with 500 mL of water and 250 mL of methanol. The dried material (60 °C, high vacuo) product was analyzed by CHN elemental analysis to give C 4.5%, H 0.9%, N 3.7%, corresponding to 0.87 mmol azido groups/g. *11-Azidoundecyl silica gel*: A stirred mixture of 0.5 g of 11-bromoundecylsilica gel (0.37 mmol/g) in 5 mL of 0.5 M solution of NaN_3 in DMSO was irradiated in a microwave reactor (CEM discover) for 5 min at 125 °C at 150 W, then cooled and worked up as above. CHN analysis: C 6.0%, H 1.4%, N 1.5% which corresponds to 0.36 mmol/g of loading.
22. All synthesized compounds (**1**–**4**) were prepared using commercially available reagents, characterized by NMR and MS spectra and were >95% pure (HPLC).
23. Chiral Technologies Europe http://www.chiral.fr.
24. *General procedure for room-temperature click immobilization*: A solution of CuI in acetonitrile (1–0.01 equiv of Cu(I), see Table 1) was quickly added to a mixture of azido silica gel (1 equiv), alkyne (0.9 equiv), DIPEA (3 equiv) in degassed acetonitrile (2 mL for 100 mg of silica gel). The vial was tightly sealed and shaken (180 rpm) at an ambient temperature for 2 days. The modified silica was then filtered and washed with acetonitrile, methanol, 2% aq EDTA solution, methanol-water mixture (1:1), 10% AcOH in methanol, and pure methanol. Vacuum dried material (12 h, 60 °C) was subjected to elemental analysis. Dihydroquinine *tert*-butylcarbamate (10 mol %) was employed as an internal standard and was used for HPLC monitoring of the reaction kinetics.
25. A similar protocol was employed for the efficient immobilization of **4** onto azido-modified polystyrene materials (prepared from commercially available Merrifield resin; 4.0 mmol chloromethyl groups/g), producing a loading level of 3.32 mmol *Cinchona* ligand/g. Azido-modified polystyrenes were also reported by other groups: (a) Loeber, S.; Gmeiner, P. *Tetrahedron* **2004**, *60*, 8699; (b) Blass, B. E.; Coburn, K. R.; Faulkner, A. L.; Seibel, W. L.; Srivastava, A. *Tetrahedron Lett.* **2004**, *44*, 2153.