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Triazolo-linked cinchona alkaloid carbamate anion exchange-type chiral stationary phases: Synthesis by *click chemistry* and evaluation

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ABSTRACT

Immobilization strategy based on Huisgen 1,3-dipolar cycloaddition (*click chemistry*) of 10,11-didehydrocinchona *tert*-butylcarbamates to azido-grafted silica gels has been evaluated for preparation of novel chiral stationary phases (**CSP 1**–**3**). The resultant 1,2,3-triazole-linked CSPs were tested under various mobile phase conditions (polar organic and reversed phase mode) with a representative set of structurally diverse racemic acids including *N*-protected aminoacids, aromatic and aryloxycarboxylic acids as well as binaphthol phosphate. The chiral recognition performance of the C3-triazole-linked CSPs was found to mirror largely that of the known C3-thioether-linked CSP in terms of elution order, enantioselectivity and retention behavior. In an effort to assess the non-specific binding expressed as retention increment of these triazole-linked CSPs, the parent azidopropyl- and triazole-modified silica materials (thus not containing the chiral head ligand) were studied independently. Compared with the corresponding CSPs, the analyte retention on the azidopropyl control column was very low, and practically negligible on the corresponding triazole-modified reference column. Only minor losses in analyte retention behavior (<5%) were observed with triazole-linked CSPs after two month of continuous use with polar–organic and reversed-phase-type mobile phases, highlighting the excellent stability of the 1,2,3-triazole linker.

1. Introduction

Chiral stationary phases (CSPs) are nowadays recognized as the tools of choice for the analytical and preparative enantiomer separation of high-value chiral compounds [1–3]. Generally, CSPs are created by immobilization of an appropriate enantioselective chiral ligand (selector, SO) onto the chromatographically suitable supporting matrix. This can be achieved either by physical entrapment or coating, or, preferably, by covalent attachment [4]. Covalent immobilization strategies involve the attachment of the chiral selector head group of interest via flexible spacer units to the surface of the support by formation of a new chemical functionality. As an essential requirement suitable immobilization procedures must preserve the chemical and stereochemical integrity of the selector systems, employing mild and non-invasive linkage chemistries. A proper choice of the tether chemistry units, its lengths and linkage functionality is crucial to the overall chiral recognition performance of the resultant CSPs, as these factors control the relative orientation and the accessibility of the selector head group towards the analytes to be stereoselectively bound from a bulk solution. Wrong-designed immobilization chemistry and/or too dense ligand grafting level may enforce restrictions in the conformational flexibility of the selector molecules, compromising thus their enantioselective binding capabilities. Mismatched spacer and linkage elements may give rise also to excessive non-specific analyte–CSP interactions leading to "non-productive" retention, largely degrading the apparent enantioselectivity of the immobilized selector [5,6]. The challenges associated with selector immobilization are well known, however, dedicated studies addressing specifically these issues are rare, and only few systematic investigations on surface engineering of CSPs have been reported [7–9].

Recently, the potential of *click chemistry* [10,11] has been demonstrated for immobilization of ligands to chromatographic supports. This immobilization strategy involves Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition (CuAAC) between azido- and alkyne-functionalized supports and ligands, furnishing stationary phases with 1,2,3-triazole-linked functionalities (Fig. 1). This conjugation concept offers several advantages over established immobilization protocols, including mild coupling conditions, excellent coupling yields, convenient control of the ligand loading level, and full compatibility with a broad range of functional groups. Pioneering efforts in this direction have been reported by Finn and Frechet, who employed CuAAC immobilization protocols for the attachment of affinity ligands onto biocompatible agarose [12] and polyacrylamide-type beads [13]. The adaptation of CuAAC immobilization procedures to the preparation of separation mate-

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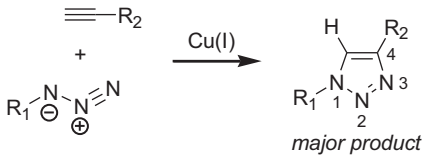


Fig. 1. Huisgen 1,3-dipolar cycloaddition (*click chemistry*).

rials based on silica supports is a recent achievement. Lei, Liang and Guo employed CuAAC methodology for the preparation of a number of simple "clicked" RP-phases involving sugars [14–16], chitooligosaccharides [17], dipeptide [18] or poly(ethylene glycol) [19] as head group. Independently Santoyo-Gonzalez and Gasparrini et al. have also prepared a set of "clicked" phases using sugars as selectors, for both hydrophilic interaction and affinity chromatography [20–22]. Boronic acid containing phases designed for separation of glycoproteins produced by *click chemistry* has been demonstrated by Bülow et al. [23]. In a recent paper, our group has reported for the first time the preparation of chiral stationary phases by CuAAC by attaching alkyne-functionalized *Cinchona* carbamates onto azido-modified silica gels [24,25]. There are also examples of "clicked" CSPs utilizing β-cyclodextrin as selector reported by group of Lei and Liang, Tan, Guo and other [26–31]. It is also worth to note that *click chemistry* protocol has been successfully adopted for the preparation of monolithic packing by Frechet and Sun [32,33].

In this contribution, we report the results of a comprehensive chromatographic study of 1,2,3-triazole-linked *Cinchona tert*-butylcarbamate-type **CSPs 1–3** in characterizing their chiral recognition profile for a representative set of acidic analytes under polar–organic and RP mobile phase conditions. We investigated also the impact of the spacer length (C3 *versus* C11-spacer) in 1,2,3-triazole-linked CSPs to assess potential changes in chiral recognition profiles enforced by restricted selector accessibility and/or induced conformational constraints. The contribution of the so-called "non-specific" adsorption increments derived from the azido- and triazole-functionalities are systematically characterized by analyzing the retention behavior of selected model analytes with appropriate reference columns. Finally, the consequences of enhanced linker-rigidity caused by the 1,2,3-triazole moiety on the global chiral recognition characteristics of *Cinchona tert*-butylcarbamate selector is assessed by comparing the chromatographic behaviors of those CSPs with the common and more flexible thioether-linked congener.

2. Experimental

2.1. Materials

10,11-Didehydroquinine and 10,11-didehydroquinidine were prepared according to the published procedure [34]. 3-Azidopropyl silica gel (**AzPrS**) and 11-azidoundecyl silica gel (**AzUS**) were prepared following a previously described protocol [24,25]. Spherical silica gel (Daiso, Japan) with a mean particle size of 5 μm, a specific surface area of 330 m^2/g and a pore size of 12 nm was used as a supporting matrix for all stationary phases. The azido group loading, calculated based on nitrogen content from CHN analysis, was 0.72 mmol/g (2.18 μmol/m^2) for **AzPrS** and 0.38 mmol/g (1.15 μmol/m^2) for **AzUS**. All other analytical grade reagents were purchased from Aldrich and used as received. Solvents and reagents: methanol (MeOH), acetonitrile (ACN) and water, ammonium acetate, triethylamine (TEA), diisopropylethylamine (DIPEA) and acetic acid (AcOH) were of analytical grade and purchased from Merck or Fluka. The pH-values of the reversed phase-type mobile phases were adjusted after mixing the solvents by addition of concentrated ortho-phosphoric acid (pH-meter control) and present therefore apparent values. The racemic test analytes were obtained from various suppliers (Aldrich, Sigma, Bachem) or prepared in-house using standard derivatization methods.

Melting points were taken on a Kofler melting point apparatus coupled with a Leica microscope and are uncorrected. NMR spectra were recorded on an Brucker Avance 400 Instrument operating at 400 MHz. Chemical shifts are reported in parts per million (ppm) relative to TMS as an internal standard. Optical rotations were measured using Perkin Elmer 341 polarimeter using a 10-cm cell at 20 °C. The IR-spectra were measured on a Brucker ITS 113v spectrometer.

2.1.1. *tert*-Butylcarbamoyl-10,11-didehydroquinine (***tBuCDDQN***)

To a solution of 10,11-didehydroquinine (5.27 g, 16.37 mmol, 1 equiv.) in 170 mL of anhydrous toluene *tert*-butylisocyanate (2.24 mL, 1.2 equiv.) was added followed by a catalytic amount (150 μL) of dibutyltin didodecanoate. The mixture was refluxed for 12 h under exclusion of moisture. After removal of volatiles *in vacuo* a brownish residue was obtained, which after flash chromatographic purification on silica gel (gradient elution with dichloromethane–MeOH, 100:0–98:2 (v/v)) yielded 5.34 g (76%) of pure product as white solid.

^{1}H NMR ($CDCl_3$): δ = 8.76 (d, J = 4.6 Hz, 1H), 8.02 (d, J = 9.3 Hz, 1H), 7.52 (d, J = 2.5 Hz, 1H), 7.39 (d, J = 4.6 Hz, 1H), 7.35 (dd, J = 2.8, 9.1 Hz, 1H), 6.44 (d, J = 8.1 Hz, 1H), 4.74 (bs, 1H), 3.96 (s, 3H), 3.56 (m, 1H), 3.14 (m, 2H), 2.84 (m, 1H), 2.62 (m, 1H), 2.51 (m, 1H), 2.16 (m, 1H), 2.06 (d, J = 2.5 Hz, 1H), 2.04 (m, 1H), 1.75 (m, 1H), 1.62 (m, 2H), 1.30 (s, 9H); ^{13}C NMR ($CDCl_3$) δ = 158.3, 148.0, 145.3, 144.5, 132.1, 128.0, 122.2, 119.4, 102.0, 88.4, 72.6, 69.0, 59.0, 57.9, 56.0, 51.0, 42.2, 29.3, 28.0, 27.2, 26.7, 25.0; IR (KBr): cm^{-1} 3250, 1712, 1623, 1511, 1476, 1364, 1268, 1227, 1109, 1032, 907, 848, 829, 717, 694; m.p. 180–182 °C, $[\alpha]_D^{20}$ − 45.0 (*c* 1.0, MeOH).

2.1.2. *tert*-Butylcarbamoyl-10,11-didehydroquinidine (***tBuCDDQD***)

***t*BuCDDQD** was prepared from 10,11-didehydroquinidine following the procedure described for *tert*-butylcarbamoyl-10,11-didehydroquinine. Yield 76%.

^{1}H NMR ($CDCl_3$): δ = 8.74 (d, J = 4.6 Hz, 1H), 8.02 (d, J = 9.6 Hz, 1H), 7.38 (m, 2H), 7.35 (d, J = 2.5 Hz, 1H), 6.49 (d, J = 4.6 Hz, 1H), 4.95 (bs, 1H), 3.95 (s, 3H), 3.19 (m, 2H), 3.09 (m, 1H), 2.83 (m, 1H), 2.73 (m, 1H), 2.54 (m, 1H), 2.27 (m, 1H), 2.19 (d, J = 2.3 Hz, 1H), 2.00 (m, 1H), 1.58 (m, 2H), 1.50 (m, 1H), 1.31 (s, 9H). ^{13}C NMR ($CDCl_3$) δ = 158.3, 147.9, 145.1, 145.0, 132.2, 122.2, 118.3, 101.7, 73.8, 69.2, 59.5, 56.0, 51.0, 50.7, 50.1, 29.3, 28.5, 28.2, 25.6, 23.1; IR (KBr): cm^{-1} 3288, 1722, 1621, 1505, 1362, 1265, 1231, 1203, 1099, 1029, 837, 824, 777, 671, 635; m.p. 145–146 °C, $[\alpha]_D^{20}$ − 15.0 (*c* 1.0, MeOH).

2.2. Preparation of chiral stationary phases (CSPs)

CSPs 1–3 (Fig. 2) and the corresponding reference phase **SP-2** (see Figs. 2 and 3) were prepared under controlled loading conditions following the reported protocol [24]. Reference column **SP-1** (0.87 mmol/g) and **SP-3** (0.70 mmol/g) were prepared according to the procedure given in Ref. [4,24]. **CSP-4** (200 μmol/g) was prepared following the protocol in Ref. [35]. The selectors loading were calculated based on the nitrogen, carbon and sulfur content determined by CHNS analysis. All stationary phases were packed into a 4 × 100 mm I.D. stainless steel HPLC column using standard slurry packing protocol (VDS Optilab, Germany).

The preparation of azidomodified silicas, **AzPS** and **AzUS**, is given in Supplementary information.

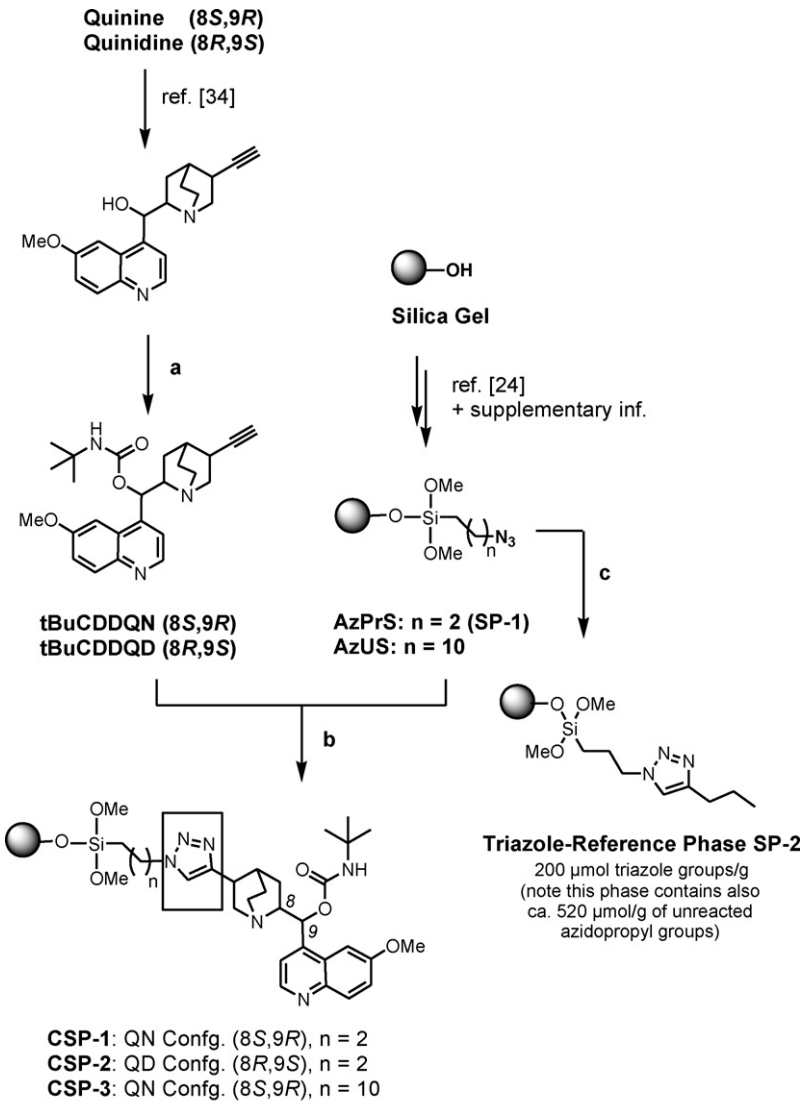


Fig. 2. Synthesis and structure of **CSPs 1–3** and **SP-2**.

*2.2.1. General procedure for preparation of **CSP 1–3** and **SP-2***

A 100 mL Erlenmeyer flask was charged with **AzPS** or **AzUS** (2.50 g, corresponding to 1.80 mmol or 0.95 mmol azide groups, respectively) and the calculated amounts of the corresponding alkynes: for **CSP-1**: ***t*BuCDDQN** 213.5 mg, 0.50 mmol; for **CSP-2**: **tBuCDDQD** 214.5 mg, 0.50 mmol; for **CSP-3**: ***t*BuCDDQN** 214.0 mg, 0.50 mmol; **SP-2**: 1-pentyne 37.0 mg, 0.54 mmol, together with diisopropylethylamine (DIPEA, 3 equiv. relative to the total amount of azido groups) in degassed acetonitrile (40 mL), followed by the addition of CuI solution in degassed acetonitrile (0.05 equiv of Cu(I) relative to the azido groups). The flask was tightly sealed, mounted on an orbital shaker and agitated at 160 rpm at ambient temperature for 2 days. The modified silicas were then isolated by filtration and washed with ACN (50 mL), aqueous EDTA solution (50 mL; 2%, w/v), MeOH–water (50 mL; 1:1, v/v), MeOH–AcOH (50 mL; 2%, v/v) and MeOH (100 mL). The resultant silica gels were dried at ambient temperature and then in *in vacuo* (12 h, 60 °C). CHN-analysis: **CSP-1**: 9.30% C, 3.56% N; (210 mmol/g) **CSP-2**: 9.60% C, 3.63% N; (220 mmol/g) **CSP-3**: 11.31% C, 2.32% N (220 mmol/g); **SP-2**: 4.85% C, 1.00% H, 2.93% N (200 mmol/g).

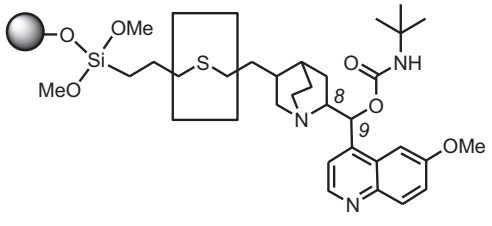


Fig. 3. Structures of **CSP 1–4**.

2.3. Chromatography

All chromatographic measurements were carried out at room temperature (25 °C) using a Merck-Hitachi HPLC system equipped

Table 1
Chromatographic retention and enantioselectivity data for analytes **1**–**20** on **CSP 1–4** under polar organic conditions using methanol.[a]

Analyte	CSP-1			CSP-2			CSP-3			CSP-4		
	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
DNB-Leu (**1**)	0.72	15.52	17.45	0.75	9.00	13.88	0.83	11.13	17.04	1.44	14.39	19.85
DNB-Ala (**2**)	0.94	8.11	15.26	0.94	5.37	11.72	1.03	6.64	15.14	1.69	8.52	19.30
DNB-Asp (**3**)	19.31	1.37	4.71	11.03	1.89	7.53	16.88	1.88	11.20	38.68	1.45	6.39
DNB-Glu (**4**)	3.51	4.98	19.00	3.06	3.39	11.21	3.62	3.99	16.62	6.90	5.33	
DNB-bPhe (**5**)	0.89	7.95	14.53	1.05	7.63	14.80	1.05	7.78	15.68	1.82	6.40	
Bz-Leu (**6**)	0.46	2.68	4.08	0.42	2.00	2.16	0.49	2.25	3.40	0.77	2.60	5.64
Bz-tLeu (**7**)	0.50	2.43	3.80	0.49	1.74	2.01	0.57	1.95	3.06	0.87	2.36	5.34
Bz-bPhe (**8**)	0.46	1.77	2.00	0.47	1.58	1.51	0.50	1.64	1.89	0.82	1.62	2.58
Ac-Phe (**9**)	0.62	1.43	1.52	0.53	1.41	1.18	0.62	1.33	1.16	0.92	1.52	2.36
Ac-Trp (**10**)	1.01	1.48	2.01	1.00	1.40	1.82	1.04	1.39	1.97	1.44	1.80	4.34
Z-Phe (**11**)	0.92	1.24	1.17	0.91	1.27	1.22	1.04	1.18	0.97	1.77	1.24	1.65
Z-Leu (**12**)	0.46	1.32	–	0.44	1.31	0.66	0.50	1.25	–	0.88	1.31	1.46
Dichloroprop (**13**)	0.82	1.32	1.80	0.82	1.48	1.84	1.12	1.33	1.74	1.72	1.25	1.66
(**14**)	0.63	1.00	–	0.66	1.11	–	0.82	1.00	–	0.99	1.12	–
Mandelic acid (**15**)	1.27	1.30	1.90	1.09	1.00	–	1.08	1.11	–	1.98	1.00	–
(**16**)	1.02	1.36	1.80	0.94	1.00	–	0.90	1.14	–	1.79	1.00	–
(**17**)	1.09	1.00	–	0.91	1.18	–	1.07	1.00	–	1.81	1.22	1.63
(**18**)	0.99	1.00	–	0.79	1.32	1.23	1.04	1.00	–	1.50	1.09	–
(**19**)	8.42	2.15	6.95	6.39	1.18	1.34	10.17	1.90	7.27	20.16	1.64	5.05
(**20**)	1.94	1.37	2.36	1.94	1.36	2.33	2.28	1.36	2.80	3.84	1.68	5.3

[a] MeOH:AcOH:NH_4OAc, 98:2:0.5 v/v/m, 1 mL/min, 254 nm detection.

with autosampler. Acetone was employed as an non-retained marker. For detailed condition see Tables 1–4.

3. Results and discussion

Huisgen 1,3-dipolar cycloaddition representing a reference reaction of *click chemistry* concept presents a highly efficient methodology for functionalizing surfaces with sensitive ligands for which mild and non-invasive conjugation conditions are imperative to preserve the structural integrity and operational functionality [36,37].

Surprisingly, so far relatively few studies have been dedicated to the development of functionalized chromatographic materials exploiting *click chemistry* principles. Most of these investigations focus on the generation of supports for bioaffinity applications (see for example optimization studies of a ligand immobilization and azide group endcapping for the preparation of adsorbents for antibody purification [38]), putting strong emphasis on material chemistry aspects or simple RP-mode separation. Little information, however, is currently available on the consequences of the triazole functionality generated during the click immobilization process on the molecular recognition characteristics of the linked ligands per se. Failure of choosing appropriate linker units may compromise the readily accessibility and/or steric adaptability the binding domains of the surface-bonded selectors for the analytes, limiting the possibility of forming stereodiscriminating analyte–selector complexes. In addition, incompatible linker units may act as non-specific stereochemically "unproductive" binding sites, competing with the "productive" enantioselective selector domains for the analytes. If such non-specific binding scenario occurs leading to an enhanced analyte retention a simultaneously apparent loss in enantioselectivity is recognized chromatographically.

Inspecting of the rich literature covering *click chemistry* it becomes evident that the chromatographic characteristics of a

Table 2
Chromatographic retention and enantioselectivity data for analytes **1**–**20** on **CSP 1–4** under polar organic conditions using acetonitrile.[a]

Analyte	CSP-1			CSP-2			CSP-3			CSP-4		
	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
DNB-Leu (**1**)	2.63	7.12	16.58	2.90	6.34	18.17	2.70	6.75	25.46	4.21	6.20	20.05
DNB-Ala (**2**)	3.32	4.37	14.75	3.72	4.39	13.78	3.49	5.06	16.88	4.48	4.28	18.00
DNB-Asp (**3**)	17.25	1.07	1.04	19.05	1.46	3.6	18.04	1.34	3.57	23.48	1.12	2.11
DNB-Glu (**4**)	19.54	2.80	12.68	21.31	3.12	14.26	21.18	2.68	14.76	28.79	2.78	15.48
DNB-bPhe (**5**)	2.06	5.57	16.30	2.35	6.38	20.15	2.13	5.53	16.43	3.43	4.02	17.43
Bz-Leu (**6**)	2.99	1.39	3.52	2.89	1.37	3.03	3.23	1.36	4.08	4.09	1.26	2.84
Bz-tLeu (**7**)	3.26	1.32	2.96	3.42	1.23	1.98	3.67	1.22	2.2	4.74	1.17	1.90
Bz-bPhe (**8**)	2.17	1.35	2.75	2.30	1.24	1.85	2.36	1.28	2.36	3.18	1.28	2.65
Ac-Phe (**9**)	4.04	1.00	–	3.91	1.12	1.12	4.71	1.00	–	4.82	1.00	–
Ac-Trp (**10**)	5.66	1.05	–	5.62	1.16	1.31	6.39	1.04	–	6.05	1.08	0.86
Z-Phe (**11**)	3.20	1.00	–	3.44	1.10	–	3.49	1.00	–	5.09	1.00	–
Z-Leu (**12**)	2.46	1.00	–	2.52	1.10	0.82	2.66	1.00	–	3.70	1.00	–
Dichloroprop (**13**)	3.19	1.16	1.54	3.60	1.27	2.29	4.21	1.17	1.62	5.29	1.12	1.27
(**14**)	2.11	1.00	–	2.30	1.11	0.97	2.5	1.06	–	2.80	1.03	–
Mandelic acid (**15**)	3.61	1.25	2.64	4.04	1.00	–	3.57	1.13	1.43	4.79	1.07	1.19
(**16**)	4.42	1.56	5.64	5.27	1.19	1.92	4.24	1.21	2.83	6.09	1.17	2.04
(**17**)	3.09	1.00	–	3.09	1.16	1.51	3.15	1.00	–	4.65	1.06	0.13
(**18**)	1.80	1.00	–	3.45	1.33	2.19	1.71	1.10	–	3.07	1.12	1.19
(**19**)	3.15	3.64	10.72	1.34	1.50	2.69	3.46	2.31	8.52	6.77	2.05	7.84
(**20**)	5.04	1.32	2.73	5.96	1.47	3.67	5.80	1.37	3.50	7.11	1.75	6.90

[a] MeCN 160 mM AcOH, 10 mM TEA, 1 mL/min, 254 nm detection.

Table 3
Chromatographic retention and enantioselectivity data for analytes **1–20** on **CSP 1–4** under reversed-phase conditions.[a]

Analyte	CSP-1			CSP-2			CSP-3			CSP-4		
	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
DNB-Leu (**1**)	3.91	12.68	24.59	3.94	7.86	17.56	5.58	8.88	22.14	6.00	13.09	23.46
DNB-Ala (**2**)	3.66	6.24	15.32	3.66	4.23	14.16	4.61	5.00	14.70	4.96	7.19	22.94
DNB-bPhe (**5**)	5.07	7.57	18.20	5.41	7.93	23.09	6.88	8.14	26.76	8.60	5.92	17.73
Bz-Leu (**6**)	2.14	2.22	6.90	1.91	1.75	4.48	2.82	1.86	5.90	2.70	2.31	5.88
Bz-tLeu (**7**)	2.18	1.91	5.31	2.02	1.54	3.27	2.94	1.62	4.61	2.76	1.95	4.60
Bz-bPhe (**8**)	2.22	1.64	3.77	2.12	1.53	3.03	2.78	1.57	3.95	3.13	1.52	2.91
Ac-Phe (**9**)	1.84	1.26	2.07	1.65	1.24	1.61	2.18	1.19	1.46	2.17	1.34	2.22
Ac-Trp (**10**)	2.84	1.38	2.46	2.60	1.25	1.65	3.44	1.26	1.99	3.67	1.49	2.82
Z-Phe (**11**)	3.96	1.19	2.25	3.73	1.19	2.29	5.64	1.14	1.75	5.91	1.19	2.07
Z-Leu (**12**)	2.37	1.24	2.07	2.16	1.21	1.58	3.23	1.18	1.66	3.39	1.24	1.98
Dichloroprop (**13**)	3.46	1.23	1.84	3.43	1.33	2.51	6.11	1.28	2.62	5.33	1.19	1.16
(**14**)	2.59	1.08	–	2.61	1.11	0.88	3.70	1.08	0.56	3.22	1.10	–
Mandelic acid (**15**)	2.97	1.07	–	2.67	1.00	–	3.30	1.00	–	3.47	1.00	–
(**16**)	2.70	1.13	1.13	2.46	1.00	–	2.88	1.07	0.43	3.59	1.00	–
(**17**)	3.20	1.00	–	3.02	1.00	–	3.95	1.00	–	4.14	1.11	1.33
(**18**)	3.37	1.00	–	2.86	1.12	0.97	4.36	1.00	–	3.97	1.08	–
(**19**)	46.58	1.62		33.76	1.00	–	79.02	1.37	4.97	76.29	1.27	3.39
(**20**)	8.91	1.26	2.24	9.64	1.24	2.00	15.05	1.22	2.55	17.80	1.46	3.17

[a] MeOH, NH_4OAc aq, 25 mM, 80:20, pH_a 5.85, 1 mL/min, 254 nm detection.

1,2,3-triazole linker moiety bound to silica is only preliminary discussed [24,25]. For this reason we study here in detail the impact of the 1,2,3-triazole-linker in contrast to the well known thioether linker of quinine and quinidine *tert*-butylcarbamate type CSPs (commercialized under names Chiralpak AX-QN and AX-QD) [39–45].

As outlined in Fig. 2 the synthesis of **CSP 1–3** is straightforward applying a *click chemistry* via the immobilization of 10,11-didehydroquinine or quinidine *tert*-butylcarbamates to two types of azido-modified silica differing in tether length: **AzPrS** with a three carbon or **AzUS** with an eleven carbon chain (Fig. 3). 1,3-Dipolar Huisgen cycloaddition was promoted using CuI (5 mol%) and DIPEA in degassed acetonitrile at room temperature according to a recently published protocol [24]. Similarly, the non-chiral reference column **SP-2** was prepared using 1-pentyne and 3-azidopropylsilica gel (**AzPrS**). For this study, moderate loading density of ~200 μmol/g of selectors has been used and we could demonstrate that our CuAAC protocol offers full control over the loading level due to the stoichiometrically working *click chemistry* concept [24]. The yield of immobilization of selectors at low and moderate loading density was practically quantitative both on analytical and preparative scale, making this procedure economic in respect to the amount of selector consumed. Kinetics of the immobilization and loading density has been easily monitored by elemental analysis. The remaining copper salts were removed from silica materials by extensive washing with EDTA and AcOH in MeOH.

3.1. Chromatography

For the chromatographic evaluation of the CSPs a set of 20 acidic analytes has been selected, covering different classes of acidic compounds: *N*-protected aminoacids (**1–12**), aryloxyacids (**13–14**), aromatic carboxylic acids (**15–19**) and binaphthol phosphate (**20**). The structures of analytes are given in Fig. 4. Three fundamentally different mobile phase compositions have been employed,

Table 4
Non-specific adsorption of analytes **1–20** on phases **SP 1–2**.

Analyte	k_1 Polar–organic[a]		k_1 Polar–organic[b]		k_1 Reversed phase[c]	
	SP-1	**SP-2**	**SP-1**	**SP-2**	**SP-1**	**SP-2**
DNB-Leu (**1**)	0.11	0.03	0.51	0.21	1.05	0.46
DNB-Ala (**2**)	0.14	0.03	1.05	0.39	0.98	0.37
DNB-Asp (**3**)	0.18	0.04	0.42	0.08	nd	nd
DNB-Glu (**4**)	0.16	0.02	1.27	0.41	nd	nd
DNB-bPhe (**5**)	0.09	0.03	0.25	0.14	0.96	0.46
Bz-Leu (**6**)	0.09	−0.02	0.77	0.31	0.63	0.22
Bz-tLeu (**7**)	0.08	0.00	0.79	0.33	0.71	0.25
Bz-bPhe (**8**)	0.06	0.00	0.51	0.24	0.6	0.24
Ac-Phe (**9**)	0.09	0.01	1.5	0.49	0.55	0.15
Ac-Trp (**10**)	0.11	0.02	1.78	0.56	0.67	0.2
Z-Phe (**11**)	0.13	0.03	0.61	0.25	0.99	0.4
Z-Leu (**12**)	0.04	−0.02	0.4	0.19	0.6	0.24
Dichloroprop (**13**)	0.16	0.02	0.8	0.37	1.02	0.41
(**14**)	0.09	0.02	0.55	0.27	0.69	0.28
Mandelic acid (**15**)	0.14	0.03	0.8	0.34	0.62	0.19
(**16**)	0.13	0.02	0.87	0.37	0.66	0.2
(**17**)	0.11	0.02	0.54	0.24	0.69	0.25
(**18**)	0.23	0.08	0.21	0.03	0.98	0.38
(**19**)	0.28	0.10	0.15	0.04	1.72	0.57
(**20**)	0.47	0.18	2.33	0.91	2.5	1.03

[a] MeOH:AcOH:NH_4OAc, 98:2:0.5 v/v/m.
[b] MeCN 160 mM AcOH, 10 mM TEA.
[c] MeOH, NH_4OAc aq, 25 mM, 80:20, pH_a 5.85.

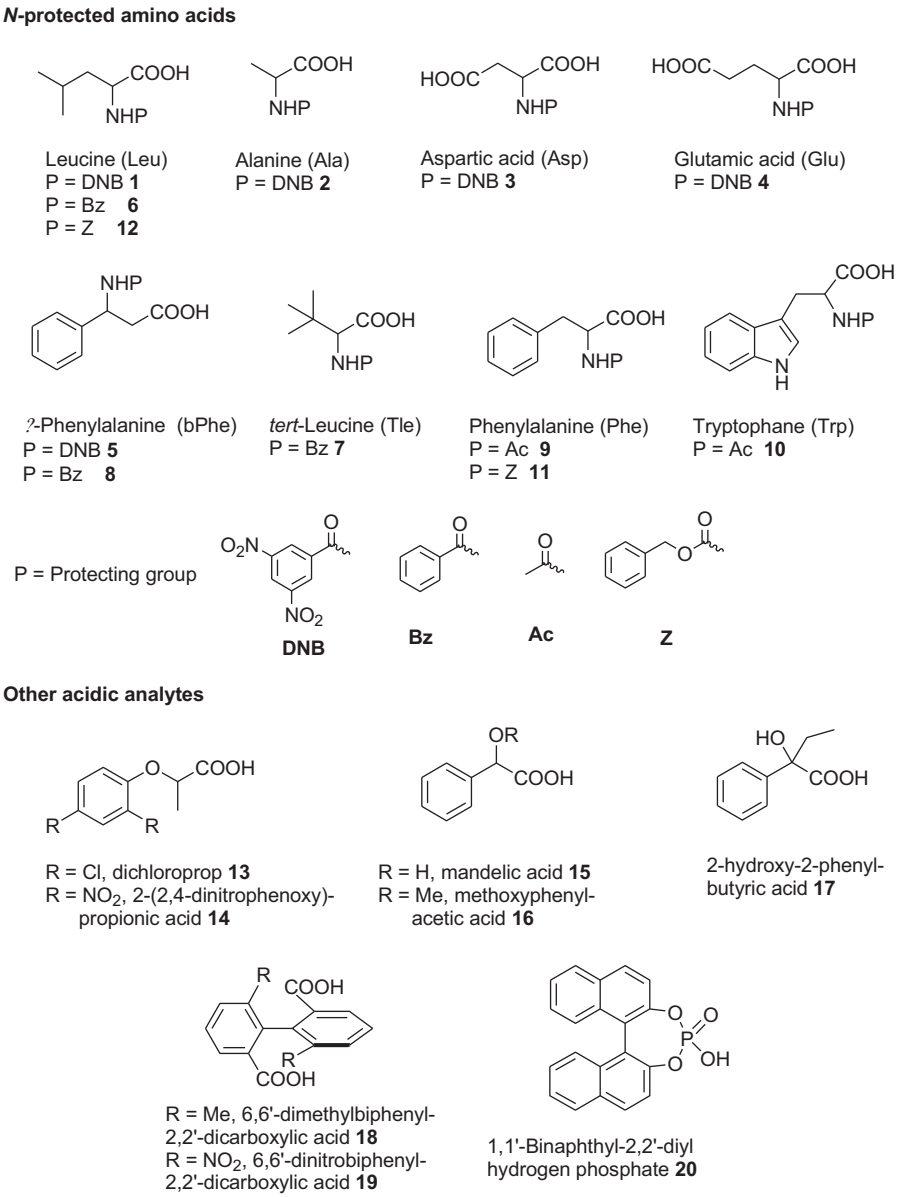


Fig. 4. Structure of analytes **1**–**20** used in this study.

including: polar organic (MeOH:AcOH:NH_4OAc, 98:2:0.5 v/v/m) acetonitrile (MeCN, 160 mM AcOH, 10 mM TEA) and reversed phase (MeOH, NH_4OAc aq, 25 mM, 80:20, pH_a 5.85).

All 1,2,3-triazole-linked phases **CSP 1**–**3** were enantioselective for standard analytes DNB-leucine, Bz-aminoacids and dichloroprop under all conditions studied. As expected, all examined chiral stationary phases (**CSP 1**–**4**) showed highest enantioselectivity (α) under polar organic mode, whereas the use of acetonitrile or reversed phase lowered the selectivity. This is in agreement with the former observations for *Cinchona* carbamate type CSPs [39–43] and confirms that similar recognition mechanisms are active under a particular operating mode. Interestingly the level of selectivity for the clicked-phases (**CSP 1**–**3**) is similar as compared to the reference thioether linked phase (**CSP-4**)[35] (*vide supra*). Comparison of quinine and its pseudoenantiomeric quinidine derived CSPs (**CSP-1** *versus* **CSP-2**) reveals that in most cases a comparable level of selectivity is retained, with expected reversal of elution order of the enantiomers. In acetonitrile, the observed selectivity (including reversed elution order) is almost identical for both quinine and quinidine selectors. Under polar organic and reversed phase, the selectivity, however, is not equal, being usually higher for most analytes on the quinine-derived phase. It is worth to note, that only the quinine phase (**CSP-1**) was able to discriminate mandelic acid (**15**) whereas the quinidine based phase (**CSP-2**) showed slightly better selectivity towards aryloxycarboxylic acids (**13**–**14**). These small differences originated from the actual diastereomeric although often called pseudoenantiomeric relationship of the quinine/quinidine pair [39,40].

3.1.1. Impact of tether length – C3 versus C11 of the immobilized QN carbamate ligand

As anticipated tether length may have impact on the overall performance of a CSP. For example Pirkle has demonstrated that shortening the tether from C11 to C3 of a tailor made CSP for Naproxen separation resulted in substantial increase of enantioselectivity [46]. Similarly, a beneficial effect on enantioselectivity of arylcarbinols separation on diphenylethanediamine based CSP in shortening the tether from C11 to C5 has been observed [47]. However, a generalization should not be made along that line as

e.g. the topologically chiral knotanes were better separated on the C-11 spaced selector [48].

For the present paper **CSP-1** and **CSP-3** containing the same amount of selector (head group) but differing in the length of tether C3 (**CSP-1**) and C11 (**CSP-3**) have been made available. Both phases showed similar profile of enantioselectivity for all analytes under all conditions studied (for data see Tables 1–3). This is to be expected for polar–organic modes (methanol and acetonitrile) which favour the ion-exchange mechanism. Surprisingly, comparable level of enantioselectivity has also been observed for the longer tether **CSP-3** under reversed phase conditions, despite a significant increase of the retention of tested analytes. Presumably, even under this condition ion-pairing/ion-exchange process involving electrostatic interaction of positively charged quinuclidine nitrogen with negatively charged carboxylates within the binding pocket of selector is of primary importance for the enantioselectivity.

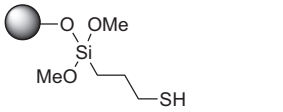
Additional interactions involving hydrogen bonding, van der Waals and hydrophobic forces will support the overall observed chiral discrimination process. A comparison shows, in line with other reports, that a shorter tether (usually also synthetically more easily accessible) may satisfy better steric demands between the surface bonded selector and low-molecular weight (small) selectands in facilitating successful enantiomer discrimination. In this context the introduction of the longer tether does not provide any additional benefits, with possible exceptions of only larger selectand molecules. In the light of this finding we decided to use 3-azidopropylsilica (**AzPS**) and corresponding CSPs as suitable phases for further studies.

3.1.2. Non-specific adsorption on plain azido- and 1,2,3-triazolo-modified support material

The 1,2,3-triazole ring (see Fig. 1) behaves as a stable, quasi-aromatic heterocycle. However the presence of three consecutive nitrogen atoms conjugated with carbon-carbon double bond results in non-equal electron distribution along the five-membered ring making the nitrogens 2 and 3 weak hydrogen acceptors in contrast to the carbon 5 which can serve as weak hydrogen donor due to its polarization (dipole moment of 1,2,3-triazoles ca. 5 D) [49]. In this context Sharpless reported about hydrogen bonding of 1,2,3-triazole-based acetylcholine esterase inhibitor in complex with AChE enzymes [50].

Similarly to the 1,2,3-triazoles the polarized azido group exhibits also strong dipole moment (e.g. 2.17 D for CH_3N_3 compared to 1.26 D for CH_3SH). Therefore the remaining azides of the pre-activated silica 0.72 mmol/g for AzPrs and 0.38 mmol/g for AzUS can also participate in electrostatic, including dipole–dipole interactions with the selectand. Thus, it becomes important for the interpretation of the retention mechanism to elucidate the non-stereospecific contributions of these groups in the course of the overall seen chromatographic enantiomer separation. For probing the magnitude of these effects the azidopropyl silica (**SP-1**) and the corresponding 4-propyl-1,2,3-triazole silica gel (**SP-2**, obtained in the reaction of **SP-1** with 1-pentyne) were compared with the mercaptopropyl silica (**SP-3**) serving as reference phase and to be known for its negligible non-specific adsorption at the given polar organic mobile phase conditions. Structures of these materials are given in Figs. 3 and 5 and the corresponding chromatographic data are given in Table 4.

In polar organic mode the azidopropyl silica (0.78 μmol/g) shows low non-specific adsorption (k_1 0.04–0.2) for compounds **1**–**17**, only for biphenyl carboxylic acids (**18**–**19**) and binaphthol phosphate (**20**) we observed a slightly higher retention (k_1 0.23–0.47). In acetonitrile, the retention of almost all analytes is much higher and lies within the range (k_1 0.21–2.33). Clear trends cannot be manifested with regards to structural parameters of the analytes. Probably acetonitrile can stabilize the dipole interaction



Thioether-Reference Phase SP-3
700 μmol thiol groups /g

Fig. 5. Stationary phases **SP 1–3** used for non-specific adsorption studies.

of azido groups towards polarized (charged) analytes as well as strengthened hydrogen bonding. Under reversed phase conditions, as expected, the non-specific adsorption is generally highest (k_1 0.6–2.5) suggesting that the CH_2 groups in concert with the relatively unpolar 1,2,3-triazole ring are responsible for it.

The reaction of 3-azidopropyl silica with 1-pentyne provided a corresponding mixed stationary phase **SP-2** containing both azides and resultant 4-propyl-1,2,3-triazole moiety (**SP-2**, 200 μmol/g) mimics somewhat the three-carbon part of the quinuclidine moiety of the *Cinchona* type selectors thus serving here as a model for tracing the non-specific adsorption. This phase showed little non-specific adsorption under all mobile phase conditions measured. Specifically, in the polar organic mode, the retention of most of the analytes (**1**–**17**) is close to zero and lies in the range k_1 0.0–0.04. The remaining azido groups are practically shielded by the 1,2,3-triazole group. In acetonitrile, the retention for most analytes was about 50% lower compared with the parent 3-azidopropyl phase **SP-1** (k_1 0.03–0.56). Even more pronounced difference in the retention was observed for the reversed phase mode (k_1 0.15–0.46), however, comparing with the polar–organic mode the retentions are still in most cases high for **SP-1**. These data provide a strong evidence that the 1,2,3-triazole is behaving "neutral" under a variety of conditions and practically do not contributed to the non-specific adsorption. Comparison of the azido- (**SP-1**) and triazole (**SP-2**) phases with a mercaptopropyl silica phase (**SP-3**) showed that the latter (non specific adsorption = 0 under all measured condition, data not shown) is devote of non-specific adsorption under all of the mobile phase modes investigated. In conclusion, the non-specific adsorption of the 1,2,3-triazole linker unit is very low under the different mobile phase conditions investigated.

3.1.3. Impact of linker type on enantioselectivity of quinine tert-butylcarbamate based CSPs

In order to study the impact of the 1,2,3-triazole linker in comparison with the well-known thioether linker **CSP-1** and **CSP-4**, both containing the same selector head group type and loading density of quinine *tert*-butylcarbamate were inspected. These linkers are similar in length but differ considerably in geometry and conformational flexibility. The 1,2,3-triazole is rigid and bulkier than the highly flexible thioether group incorporated in the position 11 of the quinuclidine core (see Fig. 2). This should make the selector–linker system more rigid and less adaptive on the one side but it may also influence to some extent the overall geometry and conformation of the immobilized quinine type selector.

For polar organic mobile phase condition both phases **CSP-1** and **CSP-4** showed quite similar enantioselectivity profile for most analytes. Only for some analytes the 1,2,3-triazole linked **CSP-1** shows slightly better enantioselectivity as can be extracted from Fig. 6.

Although the selector loading is similar, the retention factors are significantly lower for the 1,2,3-triazole phases **CSP-1** and **CSP-2** in comparison with the thioether phase **CSP-4**. Even the 11 carbon tether phase (**CSP-3**) produced slightly lower retention factors compared to **CSP-4**. This indicates that in 1,2,3-triazole phases **CSP1-3** may have some slight advantages in some cases but a generalization cannot be made at this point.

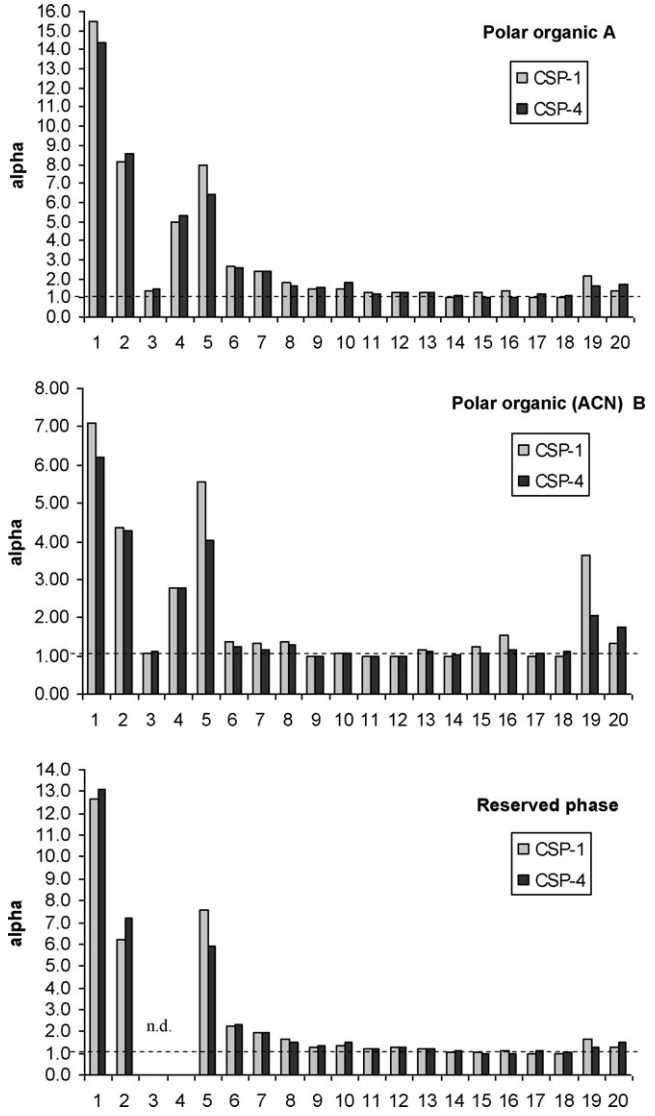


Fig. 6. Comparison of selectivity of **CSP-1** (1,2,3-triazole-linked) and **CSP-4** (thioether-linked) for enantioseparation of analytes **1**–**20** (for the structures see Fig. 3). Panel A polar organic (MeOH:AcOH:NH_4OAc, 98:2:0.5 v/v/m); panel B polar organic (MeCN 160 mM AcOH, 10 mM TEA); panel C reversed-phase (MeOH, NH_4OAc aq, 25 mM, 80:20, pH_a 5.85); 1 mL/min, 254 nm detection; n.d. = not determined.

In acetonitrile almost identical enantioselectivities have been observed for **CSP-1** and **CSP-4** (Fig. 6) but in the reversed phase mode **CSP-1** showed lower enantioselectivity as the thioether linked phase **CSP-4**. For some analytes the 1,2,3-triazole linked phase **CSP-1** is slightly superior e.g. for (**5**) and (**19**). The most notable differences, however, have been observed for the separation of mandelic acid (**15**) and its analogue methoxyphenylacetic acid (**16**). These compounds are not separated on thioether linked phase **CSP-4** in polar organic and reversed phase (and only poorly in acetonitrile) but they are baseline resolved on 1,2,3-triazole linked **CSP-1**. Under polar organic mode acids **15** and **16** showed selectivity of 1.30 and 1.36, whereas in acetonitrile 1.25 and 1.56 respectively [see also 25]. It is worth to note that the pseudoenantiomeric quinidine 1,2,3-triazole linked phase **CSP-2** is non-stereoselective for these analytes. On the other hand 2-hydroxy-2-phenylbutyric acid (**17**) is only separated on thioether linked phase which corroborates the subtle effects of the immobilization chemistry of a chiral head group on the overall observed enantioselectivity. Essentially also the tether element becomes part of the entire chiral selector moiety.

4. Conclusion

The immobilization strategy based on Huisgen 1,3-dipolar cycloaddition (*click chemistry*) of 10,11-didehydroquinine or quinidine *tert*-butylcarbamates to azido-grafted silica gels has been evaluated as a practical tool for the preparation of weak anion exchange type chiral stationary phases (CSPs). The resultant 1,2,3-triazole-linked CSPs show chiral recognition ability on the similar level as compared with the known C3-thioether-linked CSP either in terms of elution order, enantioselectivity and retention behavior. Similar observations were made for a CSP having the selector attached via a flexible C11 long 1,2,3-triazole-spacer, indicating that the inherent chiral recognition process of the *Cinchona* binding sites remains essentially unaffected. Assessment of the non-specific retention causing increments of 1,2,3-triazole-linked CSPs and the parent azidopropyl- and 1,2,3-triazole-modified silica materials revealed that it is low and in most cases negligible. The CSPs are chemically stable and allow operations in polar–organic and hydro-organic modes. Only minor losses in analyte retention behavior (<5%) were observed with 1,2,3-triazole-linked CSPs after two month of continuous use with polar–organic and reversed-phase-type mobile phases, highlighting their excellent stability. Importantly, the triazole-linkage created by the *click* immobilization protocol is non-invasive to the chiral recognition events with very little contribution to non-specific retention.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.01.031.

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