

Polysaccharide derivatives as useful chiral stationary phases in high-performance liquid chromatography*

Xiaoming Chen¹, Chiyo Yamamoto², and Yoshio Okamoto^{1,‡}

¹*EcoTopia Science Institute, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan;* ²*Department of Applied Chemistry, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan*

Abstract: The chromatographic separation of enantiomers using chiral stationary phases (CSPs) has significantly advanced. The esters and carbamates of polysaccharides coated on silica gel have been extensively studied and widely used as CSPs for high-performance liquid chromatography (HPLC). In order to overcome the strict solvent limitation on these coated CSPs, the preparation of a new generation of CSPs consisting of immobilized polysaccharide derivatives has become increasingly important. The universal solvent compatibility of the new CSPs provides flexibility in both analytical and preparative chromatographies.

Keywords: polysaccharide; chirality; chiral stationary phase; chiral separation; HPLC.

INTRODUCTION

It has been widely accepted that a pair of optically pure enantiomers may exhibit quite different bioactivities, pharmacological and toxicological behaviors, etc., and therefore, their preparation and analysis have been becoming increasingly important in many fields of science dealing with drugs, natural products, intermediates, and agrochemicals [1]. Particularly, in the pharmaceutical industry, the worldwide sales of chiral drugs in single-enantiomer forms increased annually from 27 % (U.S. \$74.4 billion) in 1996, 29 % (1997), 30 % (1998), 32 % (1999), 34 % (2000), 38 % (2001) to an estimated 39 % (U.S. \$151.9 billion) in 2002 [2]. In addition, in 9 of the top 10 drugs in the world market in 2003 and 2004, the active ingredients are chiral, and more than a half of them are commercially available as single enantiomers [3,4]. The economic interests are obvious and essential driving forces in the development of advanced technology for the separation of chiral drugs.

For more than two decades, chromatographic techniques, such as gas chromatography (GC) [5,6], high-performance liquid chromatography (HPLC) [7–10], supercritical fluid chromatography (SFC) [11,12], and capillary electrochromatography (CEC) [13] have been extensively developed for the separation of enantiomers. HPLC has become essential for the research and development of chiral drugs [14,15]. In particular, the direct separation of enantiomers by chiral stationary phases (CSPs) has been recognized to be the most advantageous one not only for determining the optical purity of enantiomers, but also for obtaining optical isomers on a large scale.

With the constant development of the methodology and applications in chromatographic enantio-separations, many CSPs have appeared for HPLC including proteins [16], oligosaccharides [17], polysaccharides [8–10,18–21], antibiotics [22], helical synthetic polymers [21], and low-molecular-weight

*Paper based on a presentation at the 12th International Conference on Polymers and Organic Chemistry 2006 (POC'06), 2–7 July 2006, Okazaki, Japan. Other presentations are published in this issue, pp. 1471–1582.

‡Corresponding author: Tel.: +81-52-789-4600; Fax: +81-52-789-3188; E-mail: okamoto@apchem.nagoya-u.ac.jp

compounds [23]. Among them, the polysaccharide derivatives, such as the cellulose esters and phenyl-carbamates of cellulose and amylose, exhibit a unique chiral recognition for a broad range of chiral compounds and have been widely used as CSPs for HPLC. For the resolution of about 500 test racemates, about 80 % of them have been successfully resolved on only two kinds of polysaccharide derivative-based CSPs [21] and 70 % of the CSPs used for preparative purposes are derived from polysaccharide derivatives [9,14].

MAIN POLYSACCHARIDE DERIVATIVES

The modified polysaccharides show much better chromatographic and enantioselective properties than the native polysaccharides themselves. Until now, we have prepared about 200 kinds of polysaccharide derivatives based on different polysaccharides including cellulose, amylose, chitin, chitosan, galactosamine, curdlan, dextran, xylan, and inulin (Fig. 1). These derivatives have been coated on a macroporous silica gel as CSPs [24,25], and their chiral recognitions were then evaluated by HPLC.

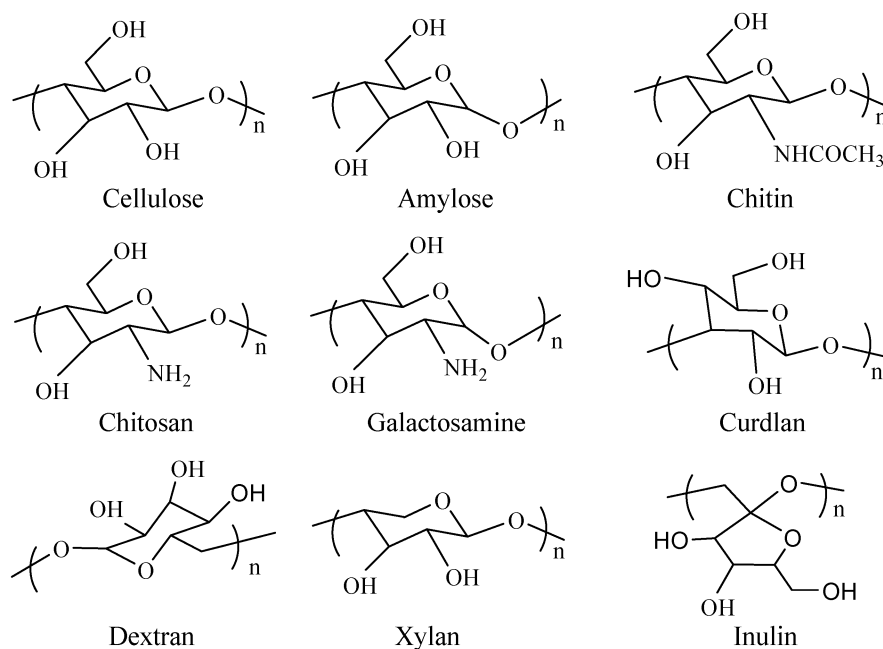


Fig. 1 Structures of the various kinds of polysaccharides.

The enantioselectivity and the elution order of the various enantiomers differed among these polysaccharides depending on the sugar units, linkage position, and linkage type. Among them, the derivatives of cellulose and amylose usually exhibit higher recognition abilities than the others, although it depends on the structure of a specific racemate. At present, more than 10 polysaccharide-based CSPs based on cellulose and amylose are commercially available. These CSPs have been widely used for the determination of the enantiomer excess (ee). Figure 2 shows the proportion of the methods used for the determination of the ee published in *Tetrahedron Asymmetry* (2003) and *Journal of the American Chemical Society* (2005). The figures in parentheses represent the number of counted papers in the years 2003 and 2005. Among these methods, about 54 % (2003) and 68 % (2005) were done by chiral HPLC. Also, more than 85 % (2003) and 90 % (2005) of chiral HPLC was performed using polysaccharide-based CSPs. The distribution of different polysaccharide derivatives used for the determination is also included.

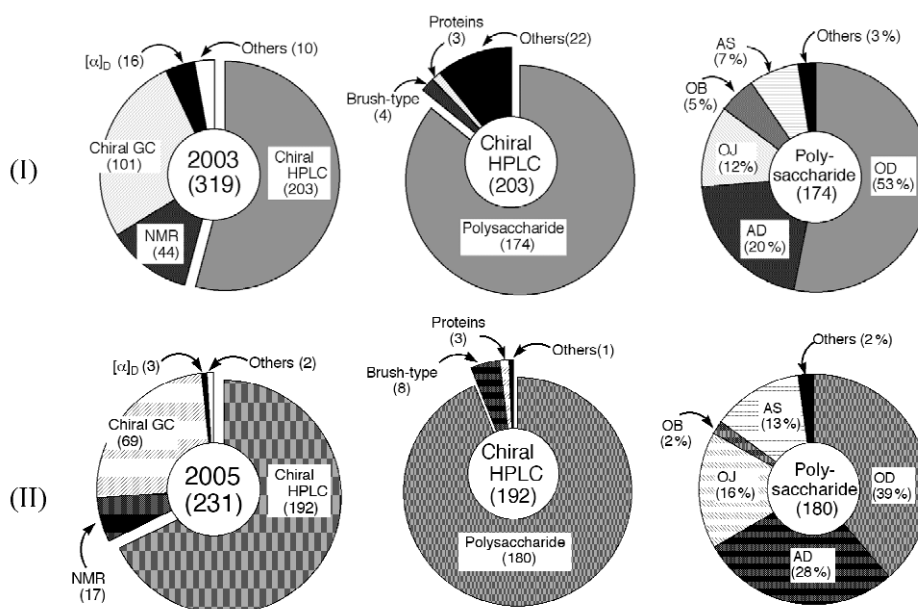


Fig. 2 Distribution of the methods used for the determination of ee appeared in (I) *Tetrahedron Asymmetry* (2003) and (II) *Journal of the American Chemical Society* (2005).

Esters of cellulose

The first practical CSP derived from polysaccharides is the microcrystalline cellulose triacetate (CTA-I) (**1**, Fig. 3) in its pure polymer form developed by Hesse and Hagel in 1973 [26]. Since then, many racemates, especially nonpolar aromatic compounds and aromatic pharmaceuticals have been separated on CTA-I using a mixture of ethanol-water as the eluent [27–29]. Interestingly, the pure CTA-I exhibited different chiral recognition abilities after being coated on a silica gel. For example, the elution order of the Tröger base enantiomers on pure CTA-I was opposite that on the coated CTA-I [30,31].

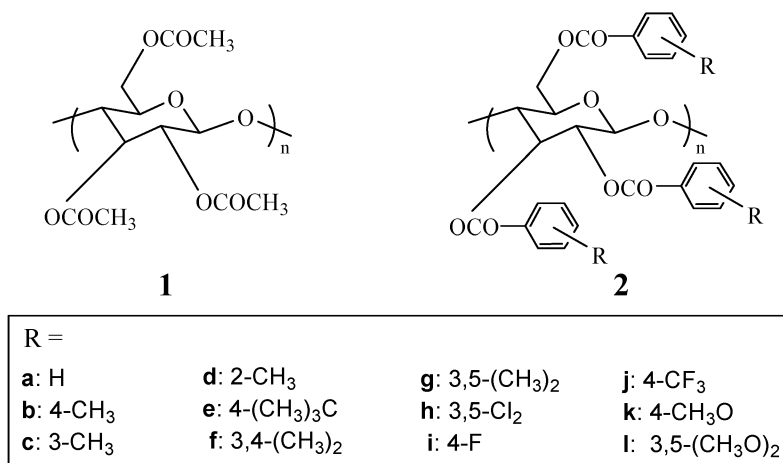
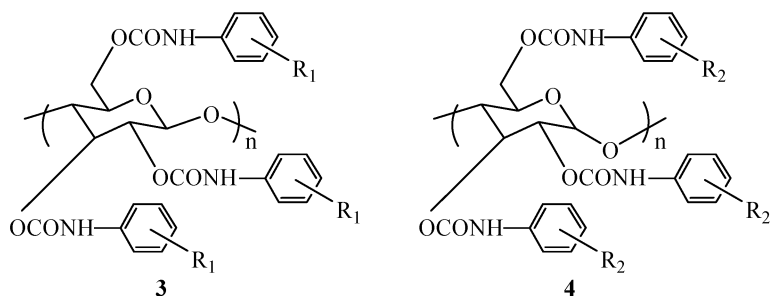


Fig. 3 Structures of the esters of cellulose.

Cellulose tribenzoates (**2**, Fig. 3) are readily obtained by reacting cellulose with the corresponding benzoyl chlorides [31,32]. The benzoate derivatives having electron-donating substituents like a methyl group, showed better chiral recognition abilities than those having electron-withdrawing substituents like a halogen. Among the benzoates, cellulose tris(4-methylbenzoate) (**2b**) showed a high chiral recognition for various racemates. The chiral recognition of **2a** was significantly influenced by the coating solvents [29,32,33]. The tribenzoates of amylose show a lower chiral recognition than the cellulose tribenzoates [32].

Phenylcarbamates of cellulose and amylose

Cellulose and amylose are easily converted to various trisphenylcarbamate derivatives (**3** and **4**, Fig. 4) when reacted with the corresponding phenyl isocyanates. Similarly, their chiral recognition abilities are significantly influenced by the substituents on the phenyl group [34–36]. Generally, the introduction of an electron-donating methyl group or an electron-withdrawing halogen at the m- and/or p-position of



R ₁ =			
a: H	l: 4-Ph ₃ C	w: 3,4-(CH ₃) ₂	ah: 2-Cl-6-CH ₃
b: 4-CH ₃	m: 4-F	x: 3,5-(CH ₃) ₂	ai: 3-Cl-2-CH ₃
c: 4-C ₂ H ₅	n: 4-Cl	y: 2,6-(CH ₃) ₂	aj: 3-Cl-4-CH ₃
d: 4-(CH ₃) ₂ CH	o: 2-Cl	z: 3,4,5-(CH ₃) ₃	ak: 4-Cl-2-CH ₃
e: 4-(CH ₃) ₃ C	p: 3-Cl	aa: 3,5-Cl ₂	al: 4-Cl-3-CH ₃
f: 4-CH ₃ O	q: 4-Br	ab: 3,4-Cl ₂	am: 3-F-4-CH ₃
g: 4-C ₂ H ₅ O	r: 4-I	ac: 2,6-Cl ₂	an: 4-F-3-CH ₃
h: 4-(CH ₃) ₂ CHO	s: 4-CF ₃	ad: 3,5-F ₂	ao: 5-F-2-CH ₃
i: 4-PhO	t: 4-NO ₂	ae: 3,5-(CF ₃) ₂	ap: 3-F-5-CH ₃
j: 4-(CH ₃) ₃ Si	u: 2-CH ₃	af: 2-Cl-4-CH ₃	aq: 3-Cl-5-CH ₃
k: 4-Ph	v: 3-CH ₃	ag: 5-Cl-2-CH ₃	ar: 3-Br-5-CH ₃
R ₂ =			
a: H	h: 4-Ph	o: 3,4,5-(CH ₃) ₃	u: 3-F-4-CH ₃
b: 4-CH ₃	i: 4-F	p: 3,5-Cl ₂	v: 4-F-3-CH ₃
c: 4-(CH ₃) ₂ CH	j: 4-Cl	q: 3,4-Cl ₂	w: 5-F-2-CH ₃
d: 4-(CH ₃) ₃ C	k: 4-Br	r: 3,5-F ₂	x: 3-F-5-CH ₃
e: 4-CH ₃ O	l: 4-I	s: 4-Cl-3-CH ₃	y: 3-Cl-5-CH ₃
f: 4-PhO	m: 3,4-(CH ₃) ₂	t: 5-Cl-2-CH ₃	z: 3-Br-5-CH ₃
g: 4-(CH ₃) ₃ Si	n: 3,5-(CH ₃) ₂		

Fig. 4 Structures of the phenylcarbamates of cellulose and amylose.

the phenyl ring improves the chiral recognitions for many racemates. In particular, the tris(3,5-dimethylphenylcarbamates) of cellulose (**3x**) and amylose (**4n**) exhibit an excellent resolving power for a wide range of racemates. The two CSPs have been commercially available as Chiralcel OD (**3x**) and Chiralpak AD (**4n**), and have been most widely utilized for enantioseparations in many fields. Interestingly, the phenylcarbamate derivatives having both an electron-donating and -withdrawing group on the phenyl moieties possess high chiral recognition abilities for many racemates [37–40]. For the cellulose phenylcarbamates, substitution at the *ortho*-position usually decreases the chiral recognition ability. On the other hand, the *ortho*-substituted amylose phenylcarbamates, such as **4t**, show a relatively high chiral recognition. These results may be ascribed to their different chain conformations of the tris(phenylcarbamate)s of cellulose (left-handed 3/2) and amylose (left-handed 4/3).

Cellulose tris(3,5-dichlorophenylcarbamate) (**3aa**) also shows a unique chiral recognition ability. However, the coated **3aa** is not stable in hexane containing 10–20 % 2-propanol. This defect can be overcome by chemical immobilization of **3aa** on a silica gel [41]. Nevertheless, **3aa** is insoluble in polar solvents, such as alcohols, acetonitrile, and water. Recently, we found that the coated **3aa** may show a high chiral recognition when using alcohol as the eluent [42–44].

Alkylcarbamates of cellulose and amylose

Alkylcarbamates, such as the methyl- and isopropylcarbamates, show a very low chiral recognition when used as CSPs, which might be due to the lack of a regular higher-order structure for these derivatives. According to our experiments, most of the cellulose tris(phenylcarbamate) derivatives form a lyotropic liquid-crystalline phase in a high concentration and a high crystallinity under a polarizing microscope when they are cast from a solution. However, the above-mentioned alkylcarbamates do not form a lyotropic liquid-crystalline phase [35,45]. Therefore, these alkylcarbamates coated on the silica surface from a solution probably do not have an ordered structure.

We later found that the cyclohexyl- and norbornylcarbamates of cellulose (**5**, Fig. 5) and amylose (**6**, Fig. 5) exhibit a high chiral recognition for a wide range of racemates [46,47]. Their chiral recognition abilities are even comparable to **3x** and **4n**. In addition, the absence of a UV absorption above 220 nm on these cycloalkylcarbamates makes it possible to use them as CSPs for thin-layer chromatography (TLC) [46], while the phenylcarbamates of polysaccharides are difficult to use for TLC due to the UV-detection problem.

Benzylcarbamates of cellulose and amylose

As mentioned above, the methyl- and isopropylcarbamates exhibit low chiral recognition, probably due to the insufficient steric requirements to form a regular higher-ordered structure. Therefore, several tris(benzylcarbamate) derivatives of cellulose (**7**, Fig. 5) and amylose (**8**, Fig. 5) were prepared and evaluated as CSPs. Among these derivatives, the 1-phenylethylcarbamates (**7b**, **8b**) and 1-phenylpropylcarbamates (**7c**, **8c**) show high chiral recognition, while the others show poor chiral recognition [48,49]. We measured the CD spectra of these aralkylcarbamates, and intensive peaks were observed only for **7b**, **7c**, **8b**, and **8c**, which indicates that these four kinds of derivatives probably possess more regular higher-order structures than the others. In addition, the chiral recognitions of the 1-phenylethylcarbamate derivatives significantly depend on the chirality of the 1-phenylethyl group.

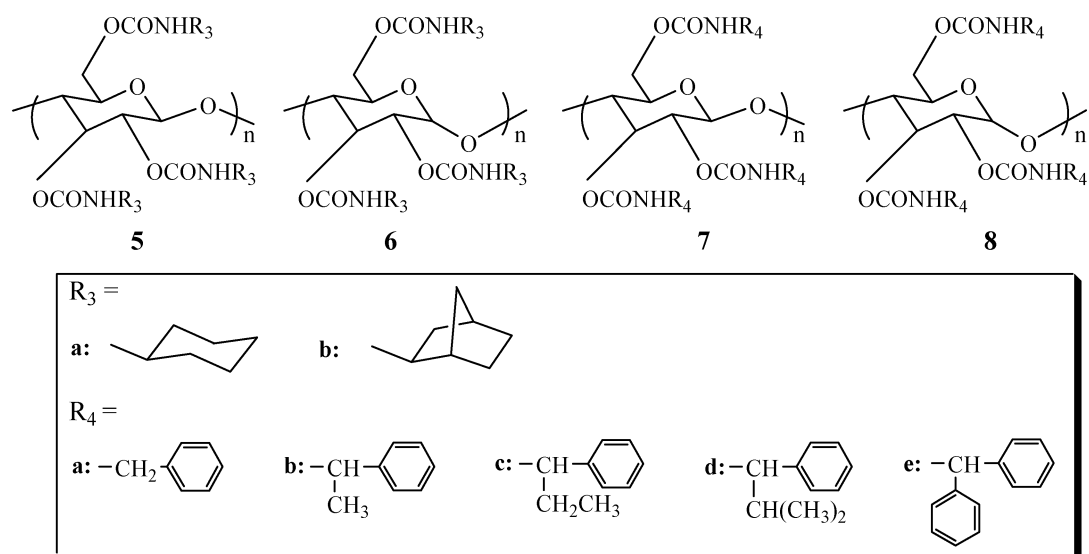


Fig. 5 Structures of the alkylcarbamates of cellulose and amylose (**5,6**), and benzylcarbamates of cellulose and amylose (**7,8**).

MECHANISM OF CHIRAL RECOGNITION

Chiral discrimination studies of polysaccharide derivatives, particularly at a molecular level, are of great importance and interest from the viewpoint of better understanding the chiral separations as well as providing an insight into developing a more efficient CSP. However, because of the number of different interaction sites with a different affinity on chiral polymers, and the difficulty determining their precise structures both in the solid state and in solution, the comprehensive elucidation of the chiral recognition mechanism on polymeric CSPs are not very easy compared to the small-molecule CSPs. Some interesting approaches have been performed to reveal the discrimination mechanism on polysaccharide derivatives using chromatographic [35], computational [50,51], and spectroscopic studies [52–55].

Basically, the high-ordered helical structures of the phenylcarbamates of cellulose and amylose are responsible for providing the high chiral recognition. It is suggested that **3x** and **4n** have a left-handed 3/2 helix [56,57] and a left-handed 4/3 helix [55], respectively. The glucose residues are regularly arranged along the helical axis, and a chiral helical groove surrounded by polar carbamate groups exists along the main chains. The polar carbamate groups are preferably located inside, while hydrophobic aromatic groups are located outside the polymer chain. Such an ordered structure seems to begin at a rather low degree of polymerization for the amylose derivatives, but may begin at a higher degree for the cellulose derivatives [58].

The polar carbamate groups on the phenylcarbamates of the polysaccharides can probably interact with a racemate via hydrogen bonding on the NH and C=O groups, and a dipole–dipole interaction on C=O. The π – π interaction between the phenyl groups of the phenylcarbamates and an aromatic group of a racemate may also play some role in the chiral discrimination.

IMMOBILIZATION OF POLYSACCHARIDE DERIVATIVES

Although the polysaccharide derivatives coated on silica gel exhibit high chiral recognition abilities for a wide range of chiral compounds, these coated CSPs are only compatible with a limited range of solvents as the mobile phase because of their coated nature, such as alkanes, alcohols, acetonitrile, and their mixtures, or aqueous solvents including alcohols or acetonitrile. This strict limitation of solvents

is a serious disadvantage since the suitable selection of various solvents is very important for attaining an efficient resolution. For a preparative large-scale separation, sufficient solubility of the target sample is essential for achieving a high productivity. Thus, versatility in the solvent selection is highly desirable. Therefore, the preparation of chemically immobilized CSPs using polysaccharide derivatives has been considered as a direct approach to confer a universal solvent compatibility on these kinds of CSPs.

About 20 years ago, we reported the first immobilization of polysaccharide derivatives on silica gel as CSPs using a diisocyanate [59], and this method has since been further developed [60]. Several immobilization methods have appeared later, such as radical polymerization [61–65], photoirradiation [66–69], enzyme-catalyzed polymerization [70], and others [71–73]. These immobilization methods have been described and compared in two review articles in great detail [74,75]. Therewith, in this article, we just briefly discuss our recently developed methods for the efficient immobilization of polysaccharide derivatives through the radical copolymerization with vinyl monomers [76–81]. In this method, a vinyl group was regioselectively or randomly introduced on the polysaccharide and the vinylated polymer was then coated onto the aminated or vinylated silica gel. The radical copolymerization was thus initiated by AIBN with the addition of a vinyl monomer to the solution.

We have introduced the vinyl groups of 2-methacryloyloxyethylcarbamate (R_6) and 4-vinylphenylcarbamate (R_8) on **3x**, **4n**, and **3aa** at the 6-positions on the glucose units by a regioselective approach to give **9–14** (Fig. 6) [78,80]. We found that **9–12** having a 30 % vinyl content can be efficiently immobilized onto the aminated and vinylated silica gel through the copolymerization with various vinyl monomers, such as styrene, isoprene, *t*-butyl acrylate, and *t*-butyl methacrylate [76–79]. However, these vinylated derivatives still exhibited somewhat lower chiral resolving abilities than the coated **3x** or **4n** due to the significant amounts of introduced vinyl groups. To further improve the chiral recognitions of the immobilized derivatives, the vinyl content on the polysaccharide was reduced to a level lower than 12 and 8 % to give **13** and **14**. We compared the chiral recognitions of **9** and **13** under the same mobile phase conditions [80]. Indeed, the chiral recognitions for most of the test racemates were improved to various degrees with the reduction of the vinyl content. The high immobilization efficiency can be maintained by using vinyl monomers or a higher temperature during the copolymerization reactions. For example, 89 % of **14** was fixed on a vinylated silica gel with the vinyl monomer 1,5-hexadiene at 80 °C for 5 h in toluene [80].

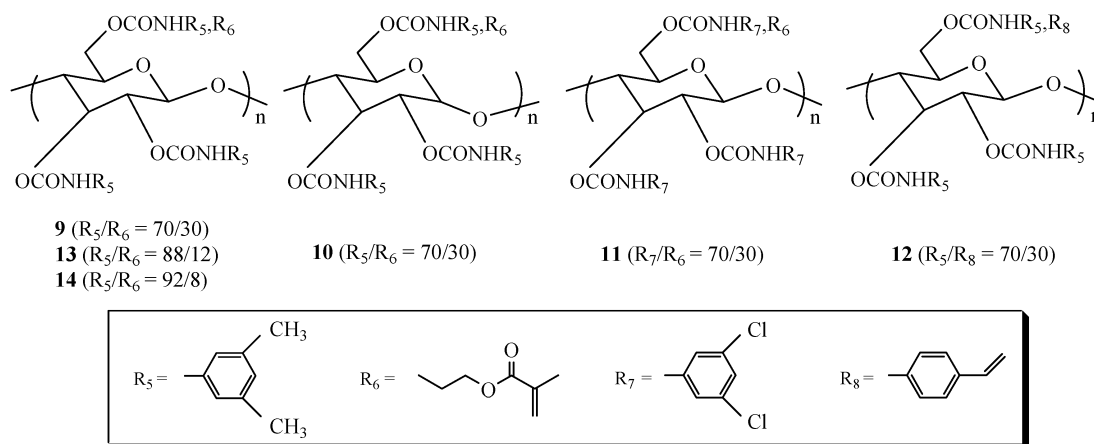


Fig. 6 Structures of the regioselectively prepared vinylated polysaccharide derivatives.

Although the regioselectively prepared derivatives can be efficiently immobilized, the regioselective approach is not quite perfect for practical purposes or large-scale production due to the time-consuming procedures. This encouraged us to develop a more practical non-regioselective method for the rapid immobilization of the chiral selector [81]. The random introduction of vinyl groups on **3x** is presented in Fig. 7. In this case, the cellulose was first dissolved in a mixture of DMA/LiCl/pyridine, then a small amount of 3,5-dimethylphenyl isocyanate and the designed amount of a vinyl reagent were simultaneously reacted with the polysaccharide. An excess of 3,5-dimethylphenyl isocyanate was then added to completely react with the residue hydroxyl groups to give the vinylated polysaccharide derivative in a one-pot method. The derivatives **15–20** (Fig. 7) with different vinyl groups or contents randomly placed at the 2, 3, or 6-positions were readily prepared using this approach. The simplicity of the non-regioselective method is obvious over the regioselective one.

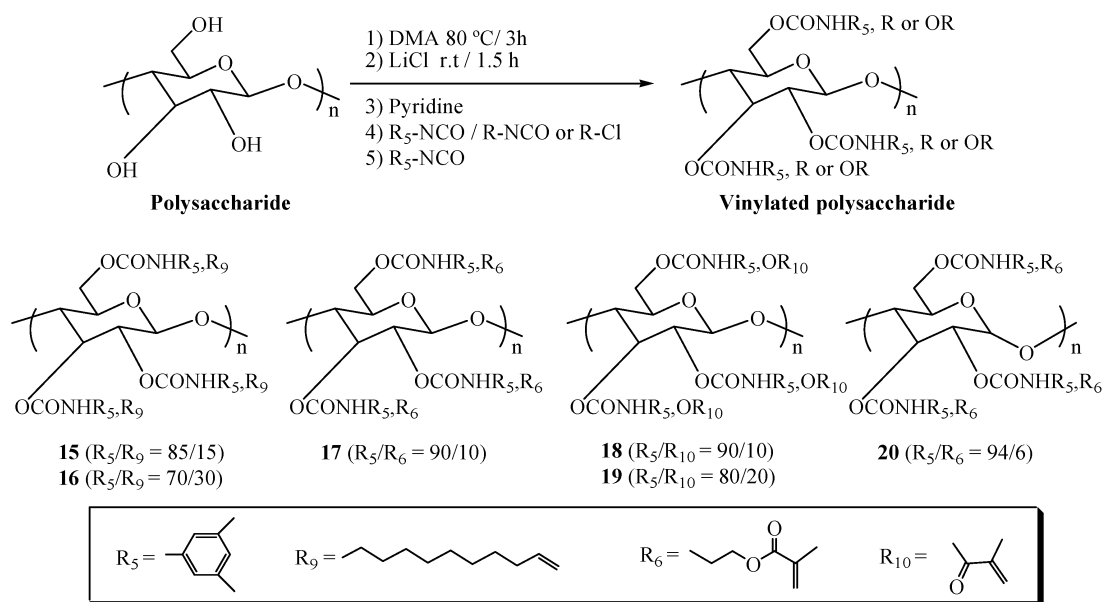


Fig. 7 Synthesis and structures of the randomly prepared vinylated polysaccharide derivatives.

We checked the chiral recognitions of the coated **15**, **17**, and **18** and found that the chiral recognitions of **3x** were influenced by the placement of the vinyl spacers (Table 1). The structures of the 10 racemates for the evaluation of the obtained CSPs are shown in Fig. 8. Briefly, the smaller molecular size of the vinyl spacer like R₁₀ may have a smaller influence on the helix structure of **3x**, thus maintaining a higher recognition. In addition, the polar groups on the vinyl spacer near the adhered chiral glucose unit as in the case of R₁₀ are superior to those that are far away from the glucose units like R₆ since a racemate may be absorbed by the polar groups to reduce the interaction with the chiral glucose units. The higher k'_1 values observed on **17** than **18** (Table 1) may also support the stronger adsorptions between the racemates and the vinyl spacer R₆. The longer spacer R₉ might hinder the interactions between the racemates and the CSP that results in a lower retention of the racemates.

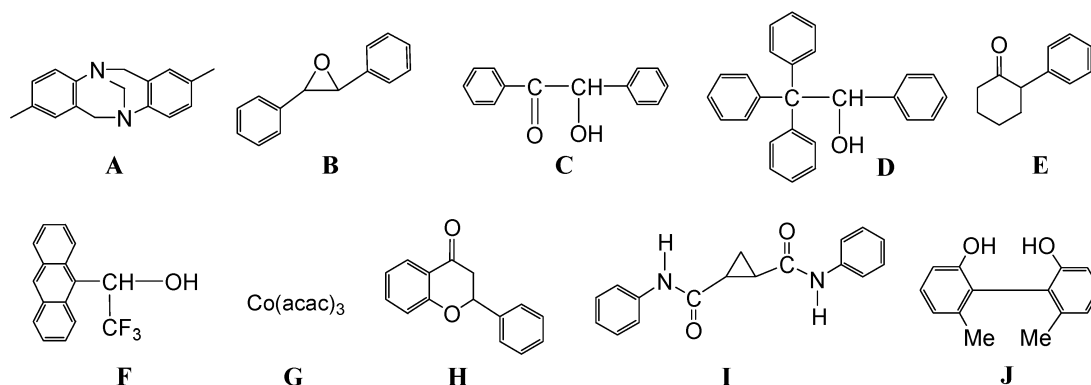
Table 1 Influence of vinyl spacers on chiral recognitions on **3x**.^a

Racemates	Derivatives							
	15 ^b		17 ^b		18 ^b		3x ^c (Chiralcel OD)	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α
A	0.38(+)	1.44	0.67(+)	1.51	0.68(+)	1.19	1.05(+)	1.26
B	0.30(-)	1.83	0.57(-)	1.70	0.45(-)	2.03	0.79(-)	2.11
C	1.02(+)	1.34	1.93(+)	1.38	1.73(+)	1.30	2.44(+)	1.57
D	0.61(-)	1.20	1.34(-)	~1	0.96(-)	1.22	1.50(-)	1.27
E	0.48(-)	1.20	0.93(-)	1.20	0.74(-)	1.21	1.22(-)	1.14
F	0.90(-)	2.26	1.67(-)	2.33	1.37(-)	2.90	2.29(-)	2.87
G	0.18(-)	~1	0.34(-)	~1	0.24(-)	~1	0.42(-)	1.13
H	0.57(-)	1.25	1.14(-)	1.25	0.88(-)	1.33	1.46(-)	1.40
I	0.46(+)	1.77	0.84(+)	1.55	0.59(+)	2.11	0.91(+)	2.63
J	2.25(-)	1.57	3.07(-)	1.78	1.47(-)	2.30	3.74(-)	1.47

^aThe signs in parentheses represent the CD detection of the first eluted enantiomer.

^bAbout 20 wt % of the derivative was respectively coated onto silica gel; column size: 250 × 2 mm; flow rate: 0.1 mL/min; mobile phase: *n*-hexane/2-propanol (90/10).

^cColumn size: 250 × 4.6 mm; flow rate: 0.5 mL/min; mobile phase: *n*-hexane/2-propanol (90/10).

**Fig. 8** Structures of the test racemates for the evaluation of CSPs.

During our experiments, we also found that the typical olefin group on **15** and **16** is significantly less effective than the methacrylate group on **17** or methacryloyl group on **18** and **19** for the radical copolymerization reaction (Table 2). On the other hand, the methacryloyl content is more sensitive than the typical olefin content on the chiral recognitions of **3x** (Table 2). For example, the chiral recognitions of **15** and **16** were slightly changed as the vinyl content increased from 15 to 30 %. However, for **18** and **19**, a considerable reduction in the chiral recognition was generally observed for most of the test racemates as the vinyl content increased from 10 to 20 %.

This non-regioselective method was also extended to amylose to give **20** bearing a 6 % R_6 , (Fig. 7). The higher efficiency observed for the immobilization of **20** (Table 2) suggests that the chain of amylose derivatives might be more flexible than that of the cellulose derivatives. The immobilized **20** only showed slightly lower chiral recognitions than the coated **4n** (Table 2).

Thus, we developed a simpler method for the sufficient immobilization of various polysaccharide derivatives as CSPs with a high chiral recognition ability. With this method, the immobilization effi-

ciency and chiral recognitions of the polysaccharide derivatives can be readily tunable by the introduced vinyl groups or the employed vinyl monomers.

Table 2 Immobilization and chiral recognitions of the randomly vinylated polysaccharide derivatives.^a

	Derivatives						
	15 ^b	16 ^b	17 ^b	18 ^b	19 ^b	20 ^b	4n ^c
	Efficiency ^d						
Racemates	33 %	60 %	87 %	78 %	91 %	92 %	(Chiralpak AD)
A	1.47(+)	1.57(+)	1.52(+)	1.34(+)	1.60(+)	1.48(+)	1.70(+)
B	1.75(-)	1.45(-)	1.53(-)	1.66(-)	1.24(-)	2.52(+)	2.81(+)
C	1.33(+)	1.26(+)	1.37(+)	1.43(+)	1.29(+)	1.14(-)	1.31(-)
D	1.06(-)	1.15(-)	~1 (-)	1.23(-)	1.17(-)	2.04(-)	2.24(-)
E	1.17(-)	1.22(-)	1.23(-)	1.24(-)	1.29(-)	~1 (-)	1.02(-)
F	2.13(-)	2.00(-)	2.24(-)	2.61(-)	2.06(-)	~1 (-)	1.39(-)
G	~1 (-)	~1 (-)	1.10(-)	~1 (-)	1.10(-)	~1 (+)	~1 (+)
H	1.21(-)	1.16(-)	1.22(-)	1.27(-)	1.13(-)	~1 (+)	1.04(+)
I	1.52(+)	1.27(+)	1.39(+)	1.96(+)	1.21(+)	2.69(+)	1.59(+)
J	2.30(-)	2.36(-)	2.75(-)	3.72(-)	3.49(-)	2.15(-)	2.22(-)

^aThe signs in parentheses represent the CD detection of the first eluted enantiomer; radical copolymerization:

[Vinyl group]/[AIBN] = 30; temp. = 80 °C; solvent = toluene; monomer = 1,5-hexadiene (monomer/polymer = 45 wt %); mobile phase: *n*-hexane/2-propanol (90/10);

^bAbout 25 wt % of the derivative was respectively coated onto the silica gel before the immobilization; column size: 250 × 2 mm; flow rate: 0.1 mL/min.

^cColumn size: 250 × 4.6 mm; flow rate: 0.5 mL/min.

^dImmobilization efficiency calculated from the recovered derivatives.

SOLVENT VERSATILITY OF IMMOBILIZED CSP

As already mentioned, the immobilized CSPs overcome the defect of solvent limitations than the coated CSPs, thus the solvent versatility of the immobilized CSPs significantly broadens the selection of solvents used as the mobile phase components or sample dissolving reagents during the HPLC. Therefore, the immobilized CSPs may enhance the chiral separations or even provide a totally new selectivity profile for some racemates with the addition of these solvents in the mobile phase, which cannot be used on the coated CSPs, such as chloroform, dichloromethane, THF, and so on.

We investigated the effect of chloroform on the chiral separations on immobilized **13** [80]. The α values (chiral recognitions) for most of the racemates were improved to different degrees with the addition of 5 or 15 % chloroform to the mobile phases. For this reason, the immobilized **13** exhibited more or less similar chiral recognitions than the coated **3x** (Chiralcel OD). A typical example is the resolution of the *trans*-stilbene oxide enantiomer. The α value on the immobilized **13** was 1.54 using the typical mobile phase of *n*-hexane/2-propanol (90/10). It then increased to 2.19 using the mobile phase of *n*-hexane/chloroform/2-propanol (85/15/1). In this case, its α value was even comparable to that (α = 2.11) on Chiralcel OD using the mobile phase of *n*-hexane/2-propanol (90/10).

Recently, we separated some compounds on the immobilized 3,5-dimethylphenylcarbamates of cellulose and amylose with the addition of chloroform or dichloromethane into the mobile phases, such as the homochirally substituted bis(dipyromethene) zinc(II) helicates [82], long chain-substituted molecular knots [83] and cyclochiral Bonnane [84]. These compounds cannot be eluted or separated on the coated CSPs using the compatible mobile phases.

CONCLUSION

In conclusion, we have shown that the variety of readily obtained polysaccharide derivatives provides broad chiral recognitions for a wide range of enantiomers, which now makes the polysaccharide derivatives the most important CSPs. The solvent versatility of the immobilized CSPs may enhance the success for chiral separations, in particular, for those racemates that cannot be resolved on the coated CSPs due to the solubility problem. Recently, the immobilized 3,5-dimethylphenylcarbamates of amylose (Chiralpak IA) and cellulose (Chiralpak IB) have appeared on the market [85,86]. We hope that the immobilized CSPs will become more and more popular in the near future, and methods developed for the efficient and rapid immobilization of polysaccharide derivatives maintaining a high chiral recognition are still attractive.

ACKNOWLEDGMENTS

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology, Daicel Chemical Company and a JSPS fellowship to X.C.

REFERENCES

1. N. M. Maier, P. Franco, W. Linder. *J. Chromatogr., A* **906**, 3 (2001).
2. H. Caner, E. Groner, L. Levy, I. Agranat. *Drug Discov. Today* **9**, 105 (2004).
3. A. Thayer. *Chem. Eng. News* **83**, 49 (2005).
4. A. M. Rouhi. *Chem. Eng. News* **82**, 47 (2004).
5. V. Schurig. *J. Chromatogr., A* **906**, 275 (2001).
6. V. Schurig. *Trends Anal. Chem.* **21**, 647 (2002).
7. D. W. Armstrong. *LC-GC Int.* **11**, 22 (1998).
8. E. Yashima, C. Yamamoto, Y. Okamoto. *Synlett* 344 (1998).
9. Y. Okamoto, E. Yashima. *Angew. Chem., Int. Ed.* **37**, 1021 (1998).
10. E. Yashima. *J. Chromatogr., A* **906**, 105 (2001).
11. K. W. Phinney. *Anal. Chem.* **72**, 204A (2000).
12. G. Terfloth. *J. Chromatogr., A* **906**, 301 (2001).
13. J. Kang, D. Wistuba, V. Schurig. *Electrophoresis* **23**, 4005 (2002).
14. H. Y. Aboul-Enein, I. W. Wainer. *The Impact of Stereochemistry on Drug Development and Use*, John Wiley, New York (1997).
15. E. R. Francotte. *J. Chromatogr., A* **906**, 379 (2001).
16. J. Haginaka. *J. Chromatogr., A* **906**, 253 (2001).
17. Z. Juvancz, J. Szejtli. *Trends Anal. Chem.* **21**, 379 (2002).
18. G. Felix. *J. Chromatogr., A* **906**, 171 (2001).
19. K. Tachibana, A. Ohnishi. *J. Chromatogr., A* **906**, 127 (2001).
20. H. Y. Aboul-Enein. *J. Chromatogr., A* **906**, 185 (2001).
21. C. Yamamoto, Y. Okamoto. *Bull. Chem. Soc. Jpn.* **77**, 227 (2004).
22. J. W. Timothy, B. F. Alton III. *J. Chromatogr., A* **906**, 73 (2001).
23. F. Gasparrini, D. Misiti, C. Villani. *J. Chromatogr., A* **906**, 35 (2001).
24. Y. Okamoto, M. Kawashima, K. Hatada. *J. Am. Chem. Soc.* **106**, 5357 (1984).
25. Y. Okamoto, M. Kawashima, K. Hatada. *J. Chromatogr.* **363**, 173 (1986).
26. G. Hesse, R. Hagel. *Chromatographia* **6**, 277 (1973).
27. G. Blaschke. *Angew. Chem., Int. Ed. Engl.* **19**, 13 (1980).
28. G. Blaschke. *J. Liq. Chromatogr.* **9**, 341 (1986).
29. T. Shibata, I. Okamoto, K. Ishii. *J. Liq. Chromatogr.* **9**, 313 (1986).
30. Y. Okamoto, M. Kawashima, K. Yamamoto, K. Hatada. *Chem. Lett.* 739 (1984).

31. A. Ichida, T. Shibata, I. Okamoto, Y. Yuki, H. Namikoshi, Y. Toda. *Chromatographia* **19**, 280 (1984).
32. Y. Okamoto, R. Aburatani, K. Hatada. *J. Chromatogr.* **389**, 95 (1987).
33. T. Shibata, T. Sei, H. Nishimura, K. Deguchi. *Chromatographia* **24**, 552 (1987).
34. Y. Okamoto, M. Kawashima, K. Hatada. *J. Am. Chem. Soc.* **106**, 5357 (1984).
35. Y. Okamoto, M. Kawashima, K. Hatada. *J. Chromatogr.* **363**, 173 (1986).
36. Y. Okamoto, R. Aburatani, T. Fukumoto, K. Hatada. *Chem. Lett.* 1857 (1987).
37. B. Chankvetadze, E. Yashima, Y. Okamoto. *J. Chromatogr., A* **670**, 39 (1994).
38. B. Chankvetadze, E. Yashima, Y. Okamoto. *J. Chromatogr., A* **694**, 101 (1995).
39. E. Yashima, C. Yamamoto, Y. Okamoto. *Polym. J.* **27**, 856 (1995).
40. B. Chankvetadze, L. Chankvetadze, S. Sidamonidze, E. Kasashima, E. Yashima, Y. Okamoto. *J. Chromatogr., A* **787**, 67 (1997).
41. Y. Okamoto, R. Aburatani, S. Miura, K. Hatada. *J. Liq. Chromatogr.* **10**, 1613 (1987).
42. B. Chankvetadze, C. Yamamoto, Y. Okamoto. *Chem. Lett.* 1176 (2000).
43. B. Chankvetadze, C. Yamamoto, Y. Okamoto. *J. Comput. Chem. High Throughput Scr.* **3**, 497 (2000).
44. B. Chankvetadze, C. Yamamoto, Y. Okamoto. *J. Chromatogr., A* **922**, 127 (2001).
45. U. Vogt, P. Zugenmaier. *Makromol. Chem. Rapid Commun.* **4**, 759 (1983).
46. T. Kubota, C. Yamamoto, Y. Okamoto. *J. Am. Chem. Soc.* **122**, 4056 (2000).
47. T. Kubota, C. Yamamoto, Y. Okamoto. *Chirality* **14**, 372 (2002).
48. Y. Okamoto, Y. Kaida, H. Hayashida, K. Hatada. *Chem. Lett.* 909 (1990).
49. Y. Kaida, Y. Okamoto. *J. Chromatogr.* **641**, 267 (1993).
50. C. Yamamoto, E. Yashima, Y. Okamoto. *Bull. Chem. Soc. Jpn.* **72**, 1815 (1999).
51. D. N. Theodorou, U. W. Suter. *Macromolecules* **18**, 1467 (1985).
52. E. Yashima, M. Yamada, Y. Okamoto. *Chem. Lett.* 579 (1994).
53. E. Yashima, M. Yamada, C. Yamamoto, M. Nakashima, Y. Okamoto. *Enantiomer* **2**, 225 (1997).
54. E. Yashima, C. Yamamoto, Y. Okamoto. *J. Am. Chem. Soc.* **118**, 4036 (1996).
55. C. Yamamoto, E. Yashima, Y. Okamoto. *J. Am. Chem. Soc.* **124**, 12583 (2002).
56. H. Steinmeier, P. Zugenmaier. *Carbohydr. Res.* **164**, 97 (1987).
57. U. Vogt, P. Zugenmaier. *Ber. Bunsen-Ges. Phys. Chem.* **89**, 1217 (1985).
58. R. Aburatani, Y. Okamoto, K. Hatada. *Bull. Chem. Soc. Jpn.* **63**, 3606 (1990).
59. Y. Okamoto, R. Aburatani, S. Miura, K. Hatada. *J. Liq. Chromatogr.* **10**, 1613 (1987).
60. E. Yashima, H. Fukaya, Y. Okamoto. *J. Chromatogr., A* **677**, 11 (1994).
61. K. Kimata, R. Tsuboi, K. Hosoya, N. Tanaka. *Anal. Meth. Instrum.* **1**, 23 (1993).
62. C. Minguillón, P. Franco, L. Oliveros, P. Lopez. *J. Chromatogr., A* **728**, 407 (1996).
63. P. Franco, C. Minguillón, L. Oliveros, P. Lopez. *J. Chromatogr., A* **791**, 37 (1997).
64. P. Franco, A. Senso, C. Minguillón, L. Oliveros. *J. Chromatogr., A* **796**, 265 (1998).
65. X. Chen, F. Qin, Y. Liu, X. Huang, H. Zou. *J. Chromatogr., A* **1034**, 109 (2004).
66. E. Francotte. PCT WO Patent 96/27615 (1996).
67. E. Francotte, T. Zhang. PCT WO Patent 97/044011 (1997).
68. E. Francotte. PCT WO Patent 97/49733 (1997).
69. E. Francotte, D. Huynh. *J. Pharm. Biomed. Anal.* **27**, 421 (2002).
70. N. Enomoto, S. Furukawa, Y. Ogasawara, H. Akano, Y. Kawamura, E. Yashima, Y. Okamoto. *Anal. Chem.* **68**, 2798 (1996).
71. X. Chen, Y. Liu, F. Qin, L. Kong, H. Zou. *J. Chromatogr., A* **1010**, 185 (2003).
72. B. Chankvetadze, T. Ikai, C. Yamamoto, Y. Okamoto. *J. Chromatogr., A* **1042**, 55 (2004).
73. T. Ikai, R. Muraki, C. Yamamoto, M. Kamigaito, Y. Okamoto. *Chem. Lett.* **33**, 1188 (2004).
74. P. Franco, A. Senso, L. Oliveros, C. Minguillón. *J. Chromatogr., A* **906**, 155 (2001).
75. T. Ikai, C. Yamamoto, M. Kamigaito, Y. Okamoto. *Polym. J.* **38**, 91 (2006).
76. T. Kubota, T. Kusano, C. Yamamoto, E. Yashima, Y. Okamoto. *Chem. Lett.* 724 (2001).

77. T. Kubota, C. Yamamoto, Y. Okamoto. *Chirality* **15**, 77 (2003).
78. T. Kubota, C. Yamamoto, Y. Okamoto. *J. Polym. Sci., Part A: Polym. Chem.* **41**, 3703 (2003).
79. T. Kubota, C. Yamamoto, Y. Okamoto. *J. Polym. Sci., Part A: Polym. Chem.* **42**, 4704 (2004).
80. X. Chen, C. Yamamoto, Y. Okamoto. *J. Chromatogr., A* **1104**, 62 (2006).
81. X. Chen, C. Yamamoto, Y. Okamoto. *J. Sep. Sci.* **29**, 1432 (2006).
82. T. E. Wood, A. C. Ross, N. D. Dalgleish, E. D. Power, A. Thompson, X. Chen, Y. Okamoto. *J. Org. Chem.* **70**, 9967 (2005).
83. M. R. Kishan, A. Parham, F. Schelhase, A. Yoneva, G. Silva, X. Chen, Y. Okamoto, F. Vögtle. *Angew. Chem., Int. Ed.* **45**, 7296 (2006).
84. A. Böhmer, J. Brüggemann, A. Kaufmann, A. Yoneva, S. Müller, F. W. Vergeer, L. F. Chi, L. DeCola, H. Fuchs, T. Kubota, X. Chen, Y. Okamoto, F. Vögtle. *Eur. J. Org. Chem.* **45** (2007).
85. T. Zhang, C. Kientzy, P. Franco, A. Ohnishi, Y. Kagamihara, H. Kurosawa. *J. Chromatogr., A* **1075**, 65 (2005).
86. T. Zhang, D. Nguyen, P. Franco, T. Murakami, A. Ohnishi, H. Kurosawa. *Anal. Chim. Acta* **557**, 221 (2006).