ELSEVIER

Journal of Chromatography A 822 (1998) 155–161

JOURNAL OF CHROMATOGRAPHY A

Short communication

Liquid chromatographic resolution of racemic amino acids and their derivatives on a new chiral stationary phase based on crown ether

Myung Ho Hyun[a,*], Jong Sung Jin[a], Wonjae Lee[b]

[a]*Department of Chemistry and Chemistry Institute for Functional Materials, Pusan National University, Pusan 609-735, South Korea*
[b]*LG Chemical Ltd., PO Box 61, Yu-Sung, Taejon 305-380, South Korea*

Received 26 May 1998; received in revised form 17 July 1998; accepted 17 July 1998

Abstract

A new high-performance liquid chromatography chiral stationary phase (CSP) was prepared by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid to silica gel. The new CSP was employed in separating the two enantiomers of various natural and unnatural racemic α-amino acids and their derivatives. All natural and unnatural racemic α-amino acids tested were resolved with reasonable separation factors on the new CSP, except for proline, which does not contain a primary amino group. Racemic α-amino acid derivatives were also tested for their separability on the new CSP. In general, *N*-monoalkyl amides of α-amino acids were resolved better than the corresponding free α-amino acids. However, *N,N*-dialkyl amides and esters of α-amino acids were not resolved as well as the corresponding free α-amino acids, except for phenylglycine derivatives. In the case of phenylglycine, both *N*-monoalkyl and *N,N*-dialkyl amides of phenylglycine were resolved better than phenylglycine itself and its ester derivative.

Keywords: Chiral stationary phases, LC; Enantiomer separation; Amino acids

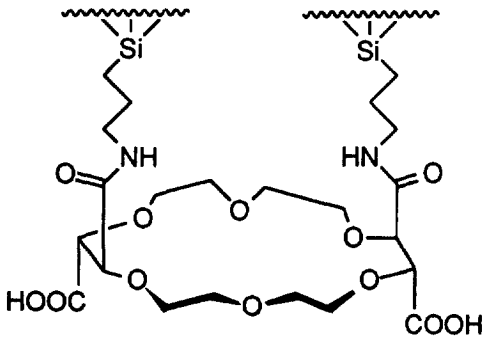
1. Introduction

The liquid chromatographic resolution of enantiomers on chiral stationary phases (CSPs) has been known as one of the most accurate and convenient means of determining the enantiomeric composition of chiral compounds [1,2]. For successful enantioseparation, various CSPs based on proteins [3], cellulose derivatives [4], cyclodextrins [5], macrocyclic antibiotics [6], low molecular mass optically active chiral molecules [7,8] and crown ethers [9,10] have been developed.

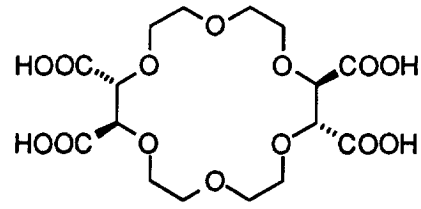
In this study, we report that a new high-performance liquid chromatography (HPLC) CSP (CSP **1**) derived from (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (**2**) (Fig. 1) can be successfully utilized in resolving various natural and unnatural α-amino acids and their derivatives. Previously, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, **2**, has been used as a chiral selector in resolving various primary amino compounds by capillary electrophoresis [11–20]. However, to the best of our knowledge, the use of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, **2**, bonded to silica gel as a CSP in HPLC has not been known, except for a study concerning the enantioseparation of racemic investigational quinolone antibacterials [21].

*Corresponding author. Fax.: 82-51-516-7421; E-mail: mhhyun@hyowon.pusan.ac.kr

0021-9673/98/$19.00
PII: S0021-9673(98)00606-2



CSP 1



2

Fig. 1. Structure of CSP **1** and (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid.

2. Experimental

2.1. Preparation of CSP 1 and column packing

CSP **1** was prepared by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, **2** (available from Aldrich), to aminopropyl silica gel. (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid, **2** (300 mg, 0.68 mmol) was refluxed in freshly distilled acetyl chloride (30 ml) for 24 h and then excess acetyl chloride was removed under reduced pressure to afford white crystalline (+)-(18-crown-6)-2,3,11,12-tetracarboxylic dianhydride (275 mg, 100% yield) via the known procedure [22].

Simultaneously, a 100-ml flask equipped with a Dean-Stark trap, a condenser and a magnetic stirring bar was charged with aminopropyl silica gel (2.5 g, Rainin AMINO 5 μm 100 Å sphere) and benzene (50 ml). The heterogeneous mixture was heated to reflux until the azeotropic removal of water was complete and then benzene was removed by rotary evaporation to afford dry aminopropyl silica gel. Aminopropyl silica gel thus dried was suspended in dry methylene chloride (20 ml) and then triethylamine (0.24 ml, 1.72 mmol) was added. To the stirred heterogeneous solution was slowly added a solution of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic dianhydride (275 mg, 0.68 mmol) in methylene chloride (5 ml) at 0°C under an argon atmosphere. The whole mixture was stirred for 2 h at 0°C and then for two days at room temperature. The modified silica gel (CSP **1**) was washed successively with methanol, water, 1 *M* HCl solution, water, methanol, methylene chloride and hexane and then dried under high vacuum. The calculation based on the elemental analyses of CSP **1** (found: C, 4.74%; H, 0.77%; N, 0.35%) and the original aminopropyl silica gel (found: C, 2.33%; H, 0.58%; N, 0.44%) showed that the loading of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, **2**, per gram of stationary phase was 0.15 mmol.

CSP **1** was slurried in methanol and packed into a 150×4.6 mm I.D. stainless-steel HPLC column using a conventional slurry packing method with an Alltech slurry packer.

2.2. Chromatography

Chromatography was performed with an HPLC system consisting of a Waters model 515 HPLC pump, a Rheodyne model 7125 injector with a 20-μl sample loop, a Younglin M720 absorbance detector (variable wavelength detector) and a Waters model 740 data module recorder. The temperature of the column was maintained at 20°C using a Julabo F30 Ultratemp 2000 cooling circulator. Chromatography samples were commercially available or prepared via the general method.

3. Results and discussion

CSP **1** was prepared by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, **2**, to silica gel via a simple two step procedure. The structure of CSP **1**

shown in Fig. 1 is believed to be the *syn*-diamide form, based on the previous study concerning the stereoselective *syn*-opening of the dianhydride by primary amino compounds in the presence of triethylamine [23]. CSP **1** thus prepared was used in resolving various natural and unnatural α-amino acids and their derivatives.

The chromatographic results for the resolution of various free racemic α-amino acids on CSP **1** are summarized in Table 1 and the representative chromatogram for the resolution of three racemic α-amino acids are shown in Fig. 2. All data shown in Table 1 and the chromatogram shown in Fig. 2 were obtained using a mixed solvent of methanol–water (80:20, v/v) containing sulfuric acid (1.0×10^{-2} M) as a mobile phase with a flow-rate of 0.5 ml/min at 20°C. Previously, it was shown that complexation of the ammonium ion ($R-NH_3^+$) inside the cavity of crown ether is essential for the chiral recognition of primary amino compounds [17]. Consequently, sulfuric acid added to the mobile phase is believed to play a role in protonating α-amino acids and in enhancing the diastereomeric complex formation of α-amino acids inside the cavity of the chiral selector of CSP **1**. Other acids, such as perchloric acid and trifluoroacetic acid, have also been tested as an acidic modifier in the mobile phase. However, sulfuric acid in the mobile phase was found to

Table 1
Separation of the two enantiomers of various α-amino acids on CSP **1**

Amino acid	k_1'[a]	k_2'[b]	α[c]	R_s[d]	Configuration[e]
Alanine	1.37	1.76	1.28	1.33	D
Arginine	1.46	2.17	1.48	1.91	D
Asparagine	1.31	1.43	1.10	0.63	D
Aspartic acid	1.51	1.84	1.22	1.25	D
Cysteine	1.32	1.44	1.10	0.30	D
Glutamic acid	1.30	1.88	1.44	1.78	D
Glutamine	1.31	1.73	1.32	1.72	D
Histidine	2.28	3.38	1.48	1.33	D
Isoleucine	0.47	0.56	1.19	0.75	D
allo-Isoleucine	0.25	0.38	1.52	1.33	D
Leucine	0.73	0.96	1.32	1.42	D
Lysine	7.43	17.06	2.30	6.00	
Methionine	1.27	1.76	1.39	2.06	D
Phenylalanine	0.88	1.30	1.48	2.31	D
Proline	0.12	0.12	1.00	0.00	
Serine	1.94	2.94	1.52	1.63	D
Threonine	0.24	0.34	1.42	1.30	L
Tryptophan	0.92	1.33	1.44	2.15	D
Tyrosine	0.84	1.21	1.44	2.00	D
Valine	0.40	0.52	1.31	1.14	D
Phenylglycine	2.07	4.66	2.25	6.46	D
4-Methoxyphenylglycine	2.04	4.40	2.16	5.31	
α-Naphthylglycine	2.53	3.68	1.45	2.67	
3,4-Dihydroxyphenylalanine (dopa)	0.92	1.32	1.43	2.40	
2-Aminobutanoic acid	0.72	0.97	1.34	2.06	
2-Aminopentanoic acid (norvaline)	0.73	0.99	1.36	1.89	
2-Aminohexanoic acid (norleucine)	0.70	0.94	1.34	1.67	D
2-Aminooctanoic acid	0.65	0.94	1.44	1.67	

Mobile phase: 80% CH_3OH in $H_2O+H_2SO_4$ (10 m*M*). Flow-rate: 0.5 ml/min. Detection: 210 nm UV. Temperature: 20°C.
[a]Capacity factor for the first eluted enantiomer.
[b]Capacity factor for the second eluted enantiomer.
[c]Separation factor.
[d]Resolution factor.
[e]Absolute configuration of the second eluted enantiomer. For blanks, the elution orders were not determined.

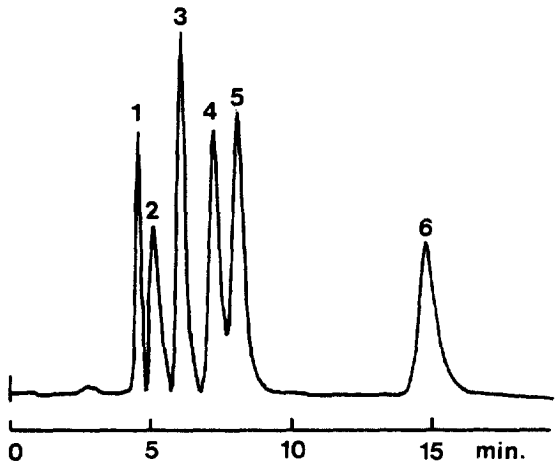


Fig. 2. Representative chromatogram for the resolution of three racemic α-amino acids on CSP **1**. Chromatographic conditions are given in Table 1. The six peaks are as follows: 1=L-leucine, 2=D-leucine, 3=L-methionine, 4=D-methionine, 5=L-phenylglycine, 6=D-phenylglycine.

afford, in general, the best resolution results. In particular, the resolution factors, R_s, were very good when sulfuric acid was used as an acidic modifier in the mobile phase. However, the reason for the good resolution with sulfuric acid as an acidic modifier in the mobile phase is not clear yet. In addition, we tried to find out the optimum composition of the mobile phase for the resolution of a broad spectrum of α-amino acids on CSP **1** by varying the methanol content of the mixed solvent, varying the concentration of the acidic modifier in the mixed solvent and changing the organic modifier in the mixed solvent from methanol to ethanol and to acetonitrile. Some chromatographic separation data for the two enantiomers of leucine and phenylglycine on CSP **1** with various mobile phase conditions are presented in Table 2. Even though each α-amino acid, including leucine and phenylglycine, responded slightly differently to the variation of the composition of the mobile phase, we concluded that the mobile phase used for the collection of the chromatographic data shown in Table 1 was the most widely applicable.

As shown in Table 1, all natural and unnatural α-amino acids tested were resolved on CSP **1** with reasonable separation factors except for proline. Proline does not contain a primary amino group. Consequently, formation of the ammonium ion (R–

Table 2
Separation of the two enantiomers of leucine and phenylglycine on CSP **1** with various mobile phase conditions

Mobile phase	Leucine			Phenylglycine		
	k_1'[a]	α[b]	R_s[c]	k_1'[a]	α[b]	R_s[c]
20% $CH_3OH+H_2SO_4$ (10 m*M*)	0.32	1.34	1.46	1.02	1.96	3.16
40% $CH_3OH+H_2SO_4$ (10 m*M*)	0.44	1.39	1.32	1.43	2.09	4.89
80% $CH_3OH+H_2SO_4$ (10 m*M*)	0.73	1.32	1.42	2.07	2.25	6.46
100% $CH_3OH+H_2SO_4$ (10 m*M*)	0.99	1.21	0.72	3.48	2.49	9.60
80% $CH_3OH+H_2SO_4$ (20 m*M*)	0.80	1.28	1.36	2.09	2.25	7.02
80% $CH_3OH+H_2SO_4$ (5 m*M*)	0.68	1.40	1.63	2.16	2.26	5.74
80% $CH_3OH+H_2SO_4$ (1 m*M*)	0.66	1.45	1.71	3.23	2.23	4.54
80% $CH_3OH+HClO_4$ (10 m*M*)	0.52	1.33	1.25	1.49	2.19	5.10
80% CH_3OH+CF_3A[d] (10 m*M*)	0.35	1.00	0.00	1.02	2.24	3.96
20% Ethanol+H_2SO_4 (10 m*M*)	0.32	1.00	0.00	0.85	1.94	3.42
40% Ethanol+H_2SO_4 (10 m*M*)	0.49	1.00	0.00	1.33	2.07	4.65
80% Ethanol+H_2SO_4 (10 m*M*)	1.94	1.09	0.32	4.84	2.04	6.58
20% $CH_3CN+H_2SO_4$ (10 m*M*)	0.19	1.00	0.00	0.49	1.92	2.48
40% $CH_3CN+H_2SO_4$ (10 m*M*)	0.21	1.00	0.00	0.45	2.14	3.04
80% $CH_3CN+H_2SO_4$ (10 m*M*)	0.46	1.06	0.40	1.63	2.09	8.93

Mobile phase: Percentage of the organic modifier in the aqueous mobile phase is given in the table. Flow-rate: 0.5 ml/min. Detection: 210 nm UV. Temperature: 20°C.

[a]Capacity factor for the first eluted enantiomer.

[b]Separation factor.

[c]Resolution factor.

[d]Trifluoroacetic acid.

NH_3^+) by protonating proline is not possible and complexation of the protonated proline inside the cavity of crown ether does not occur. In this context, no resolution of proline on CSP **1** and the shortest retention time of proline are readily inferred. The elution orders shown in Table 1 were determined by *injecting configurationally known samples*. The elution orders shown in Table 1 are quite consistent, the D-enantiomers being retained longer than the L-enantiomers, except for threonine. The resolution factors for most α-amino acids were more than 1.00, except for asparagine, cysteine and isoleucine. Interestingly, the diastereomeric mixture of isoleucine and *allo*-isoleucine with two stereogenic centers were resolved into four stereoisomers.

CSP **1** was also quite good at resolving α-amino amides and esters. Table 3 shows the resolution of some α-amino amides and esters. In order to compare the chiral recognition ability of CSP **1** for free α-amino acids and their amide or ester derivatives, the data summarized in Table 3 were collected under the same chromatographic conditions as those used for the data shown in Table 1. One interesting observation is that the 2,6-dimethylanilide derivative of alanine (tocainide), which is known to be a cardiac depressant, shows baseline resolution on CSP **1**. In general, *N*-monoalkyl amide derivatives of α-amino acids are resolved better than the corresponding free α-amino acids on CSP **1**, as shown in Table 3. However, the *N*,*N*-dialkyl amide derivatives and the ester derivatives of α-amino acids are resolved only slightly or not resolved on CSP **1**, except for phenylglycine derivatives. From these results, it is expected that the N–H hydrogen of

Table 3
Separation of the two enantiomers of some α-amino amides and esters on CSP **1**

*R*CH(NH_2)CO*Y*		k_1'[a]	k_2'[b]	α[c]	R_s[d]	Configuration[e]
R	*Y*					
CH_3	$NH(CH_2)_3CH_3$	1.60	2.25	1.41	2.34	R
(alanine)	$NHC(CH_3)_3$	1.39	1.98	1.42	2.32	R
	$NHCH_2C_6H_5$	2.58	3.57	1.38	2.33	R
	$N(CH_2CH_3)_2$	2.13	2.13	1.00	0.00	
	NH-(2,6-Dimethyl phenyl) (Tocainide)	1.90	2.22	1.16	1.31	R
	OCH_3	1.36	1.50	1.10	0.48	R
$CH(CH_3)_2$	$NH(CH_2)_3CH_3$	0.28	0.45	1.64	1.32	R
(valine)	$NHC(CH_3)_3$	0.25	0.39	1.59	1.11	R
	$NHCH_2C_6H_5$	0.46	0.68	1.48	1.50	R
	$N(CH_2CH_3)_2$	0.21	0.21	1.00	0.00	
	OCH_2CH_3	0.39	0.52	1.33	0.80	R
$CH_2CH(CH_3)_2$	$NH(CH_2)_2CH_3$	1.07	2.65	2.48	8.15	R
(leucine)	$NH(CH_2)_3CH_3$	1.03	2.80	2.71	8.30	R
	$N(CH_2CH_3)_2$	0.42	0.52	1.24	0.94	R
	OCH_2CH_3	0.86	0.86	1.00	0.00	
$CH_2C_6H_5$	$NH(CH_2)_2CH_3$	1.94	4.74	2.45	6.99	
(phenylalanine)	$NHC(CH_3)_3$	2.06	4.68	2.28	7.36	
	$N(CH_2CH_3)_2$	1.88	1.88	1.00	0.00	
	OCH_2CH_3	1.43	1.43	1.00	0.00	
C_6H_5	$NH(CH_2)_2CH_3$	1.55	3.82	2.46	7.27	R
(phenylglycine)	$NHC(CH_3)_3$	1.28	3.42	2.67	6.32	R
	$N(CH_2CH_3)_2$	1.40	4.40	3.15	9.77	R
	OCH_3	2.10	4.40	2.09	6.85	R
4-CH_3O-C_6H_5	$NHCH_3$	1.73	4.14	2.39	8.63	R
	$NH(CH_2)_2CH_3$	1.59	3.87	2.43	7.27	R
	$NHC(CH_3)_3$	1.35	3.54	2.62	7.47	R

Mobile phase: 80% CH_3OH in H_2O+H_2SO_4 (10 m*M*). Flow-rate: 0.5 ml/min. Detection: 210 nm UV. Temperature: 20°C.
[a,b,c,d,e]See the footnote to Table 1.

N-monoalkyl amide derivatives of α-amino acids play a somewhat important role in the chiral recognition. However, the exact role of the N–H hydrogen of *N*-monoalkyl amide derivatives of α-amino acids in chiral recognition is not clear yet. In the case of phenylglycine, the *N,N*-diethyl amide derivative is resolved much better than the *N*-propyl or *N-tert.*-butyl amide derivative. In addition, the resolution of phenylglycine methyl ester on CSP **1** is quite good. Consequently, the chiral recognition mechanism for the resolution of phenylglycine amides and esters is expected to be somewhat different from that for the resolution of other α-amino acid derivatives.

The mechanism of chiral recognition for the resolution of α-amino acids and their derivatives on CSP **1** is not clear yet. However, as mentioned above, complexation of the primary ammonium group ($R-NH_3^+$) formed by protonating α-amino acids under acidic conditions inside the cavity of the 18-crown-6 ring of CSP **1** is believed to be essential for the chiral recognition [17]. With the complexation of the primary ammonium group inside the cavity of the 18-crown-6 ring, additional interactions between the crown ether side groups, such as the carboxylic acid group and the α-amino acid side groups, are expected to be necessary for chiral resolution. In this instance, the side two carboxylic acid groups of the chiral selector of CSP **1** can act as steric barrier groups or as hydrogen bonding donor or acceptor groups [11]. To find out the mechanism of chiral recognition, including the exact role of the two carboxylic acid groups of CSP **1**, further studies are needed and are underway in our laboratory.

In conclusion, in this short communication, we demonstrated that CSP **1**, prepared by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, **2**, to silica gel, can be successfully employed in resolving various natural and unnatural α-amino acids and their derivatives. Other racemic compounds containing a primary amino group, such as amino alcohols, racemic amines and dipeptides are also expected to be resolved on CSP **1**. The resolution of other racemic compounds containing a primary amino group on CSP **1** is underway in our laboratory and the preliminary results show that CSP **1** is very successful in resolving a broad spectrum of other racemic compounds containing a primary amino group (the resolution of other racemic compounds on CSP **1** will be reported as soon as the study is complete). An important advantage of CSP **1** over other commercial crown ether-based CSPs (such as CROWNPAK from Daicel) is that it can be used with various mobile phases without any deterioration in its chiral recognition ability because the chiral selector of CSP **1** is bonded to silica gel covalently. For example, even 100% methanol can be used as a mobile phase for the resolution of racemic compounds on CSP **1**. Consequently, CSP **1** is expected to be more widely used than other commercial crown ether-based CSPs in resolving racemic compounds containing a primary amino group.

Acknowledgements

This work was supported by grants from the Korea Science and Engineering Foundation (97-0501-08-01-03) and from the Basic Science Research program, Ministry of Education, Korea (BSRI-97-3410).

References

[1] G. Subramanian (Ed.), A Practical Approach to Chiral Separation by Liquid Chromatography, VCH, Weinheim, 1994.
[2] E.R. Francotte, in S. Ahuja (Ed.), Chiral Separations: Applications and Technology, American Chemical Society, Washington, DC, 1997, pp. 271–308.
[3] S. Allenmark, B. Bomgren, H. Boren, J. Chromatogr. 237 (1982) 473–477.
[4] Y. Okamoto, M. Kawashima, K. Hatada, J. Am. Chem. Soc. 106 (1984) 5357–5359.
[5] T.J. Ward, D.W. Armstrong, J. Liq. Chromatogr. 9 (1986) 407–423.
[6] D.W. Armstrong, Y. Tang, S. Chen, Y. Zhou, C. Bagwill, J.R. Chen, Anal. Chem. 66 (1994) 1473–1484.
[7] W.H. Pirkle, C.J. Welch, B. Lamm, J. Org. Chem. 57 (1992) 3854–3860.
[8] M.H. Hyun, C.S. Min, Tetrahedron Lett. 38 (1997) 1943–1946.
[9] G.D.Y. Sogah, D.J. Cram, J. Am. Chem. Soc. 101 (1979) 3035–3042.
[10] T. Shinbo, T. Yamaguchi, K. Nishimura, M. Sugiura, J. Chromatogr. 405 (1987) 145–153.
[11] R. Kuhn, F. Erni, T. Bereuter, J. Hausler, Anal. Chem. 64 (1992) 2815–2820.
[12] R. Kuhn, F. Stoecklin, F. Erni, Chromatographia 33 (1992) 32–36.

[13] R. Kuhn, S. Hoffstetteruhn, Chromatographia 34 (1992) 505–512.
[14] E. Hohne, G.-J. Krauss, G. Gubitz, J. High Resolut. Chromatogr. 15 (1992) 698–700.
[15] Y. Walbroehl, J. Wagner, J. Chromatogr. A 680 (1994) 253–261.
[16] Y. Walbroehl, J. Wagner, J. Chromatogr. A 685 (1994) 321–329.
[17] R. Kuhn, C. Steinmetz, T. Bereuter, P. Haas, F. Erni, J. Chromatogr. A 666 (1994) 367–373.
[18] M.G. Schmid, G. Gubitz, J. Chromatogr. A 709 (1995) 81–88.
[19] J.-M. Lin, T. Nakagama, T. Hobo, Chromatographia 42 (1996) 559–565.
[20] Y. Mori, K. Ueno, T. Umeda, J. Chromatogr. A 757 (1997) 328–332.
[21] M.H. Hyun, J.S. Jin, W. Lee, Bull. Kor. Chem. Soc. 19 (1998) 819–821.
[22] J.-P. Behr, J.-M. Girodeau, C. Hayward, J.-M. Lehn, J.-P. Sauvage, Helv. Chim. Acta 63 (1980) 2096–2111.
[23] J.-P. Behr, J.M. Lehn, D. Moras, J.C. Thierry, J. Am. Chem. Soc. 103 (1981) 701–703.