Pergamon

Tetrahedron 55 (1999) 3883–3892

TETRAHEDRON

D/L Selective Re-binding of Saccharide-Imprinted [60]Fullerene-Bisadducts Based on a Saccharide-Boronic Acid Interaction: Development of a Molecular Imprinting Technique Useful in a Homogeneous System

Tsutomu Ishi-i, Ritsuko Iguchi, and Seiji Shinkai*

Chemotransfiguration Project, Japan Science and Technology Corporation (JST),

2432 Aikawa, Kurume, Fukuoka 839-0861, Japan

Received 11 January 1999; accepted 3 February 1999

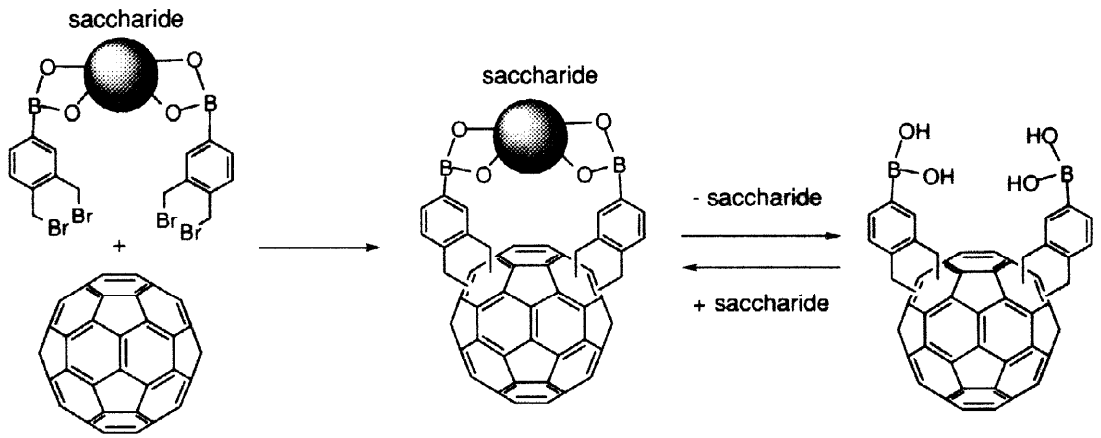
Abstract: The memory effect of the D-threitol- or L-threitol-imprinted [60]fullerene-bisadduct [(^{f}C)-**4** or (^{f}A)-**4**], which was obtained from the double addition reaction between [60]fullerene and D-threitol- or L-threitol-boronic acid 1:2 complex (D-**1** or L-**1**), was discussed. In the competitive complexation with D-threitol and L-threitol, (^{f}C)-**4** or (^{f}A)-**4** diastereoselectively rebound with the original template, D-threitol or L-threitol, respectively, affording the corresponding 1:1 complex [(^{f}C)-**3D** or (^{f}A)-**3L**]. The selectivities increased with lowering the complexation temperature: under the optimum conditions 48% diastereomeric excess was achieved. © 1999 Published by Elsevier Science Ltd. All rights reserved.

INTRODUCTION

In molecular recognition, the novel host molecule showing extremely high selectivity only with a target molecule is desired. To the best of our knowledge, however, there are few general strategies by which one can freely designed such highly guest-selective host molecules. The molecular imprinting technique is one of the most potential methods to create such molecular recognition systems with guest selectivity.[1,2] The principle idea of this technique is co-polymerization of functional vinyl monomers with crosslinking divinyl monomers in the presence of guest molecules to produce three-dimensional network polymers. It is thus expected that after removal of the guest molecules used as templates, the resulting imprinted-polymers have a memory for the template molecules. For the last few decades this technique has been of much concern, however, a few problems have been left unsolved: *e.g.*, 1) the re-binding process can be performed only in a heterogeneous system because of the insoluble property of the imprinted-polymers; thus, the estimation method of the imprinting effect has been highly limited (*i.e.* HPLC analysis), 2) the memory storage capacity is small, because some binding sites are buried in the three-dimensional network structure of polymers and only the particle surface is useful for the re-binding of guests, and 3) the total selectivity of the imprinted-polymers with the guests is canceled because of the selectivity differences between individual binding sites in the polymer

PII: S0040-4020(99)00117-9

structure. In order to overcome the problems described above, we have been searching for the new molecular imprinting technique which is useful even in a homogeneous system. In this strategy, a nano-size matrix is selected as an imprinting base of host molecule, since it is possible to create only single binding site in each nano-size space and to avoid the creation of many different binding sites. The matrix selected here should be soluble in organic solvents in order to apply it to homogeneous systems where both the imprinting process and the estimation process are more reliable and should be covered with a plenty of reactive groups which are useful for the immobilization of functional groups as binding sites. [60]Fullerene and its homologues are one of such candidates for the matrix, judging from the sizes (>0.7 nm), the moderate solubility in organic solvents, and a plenty of the reactive carbon-carbon bonds on the surfaces of fullerenes.



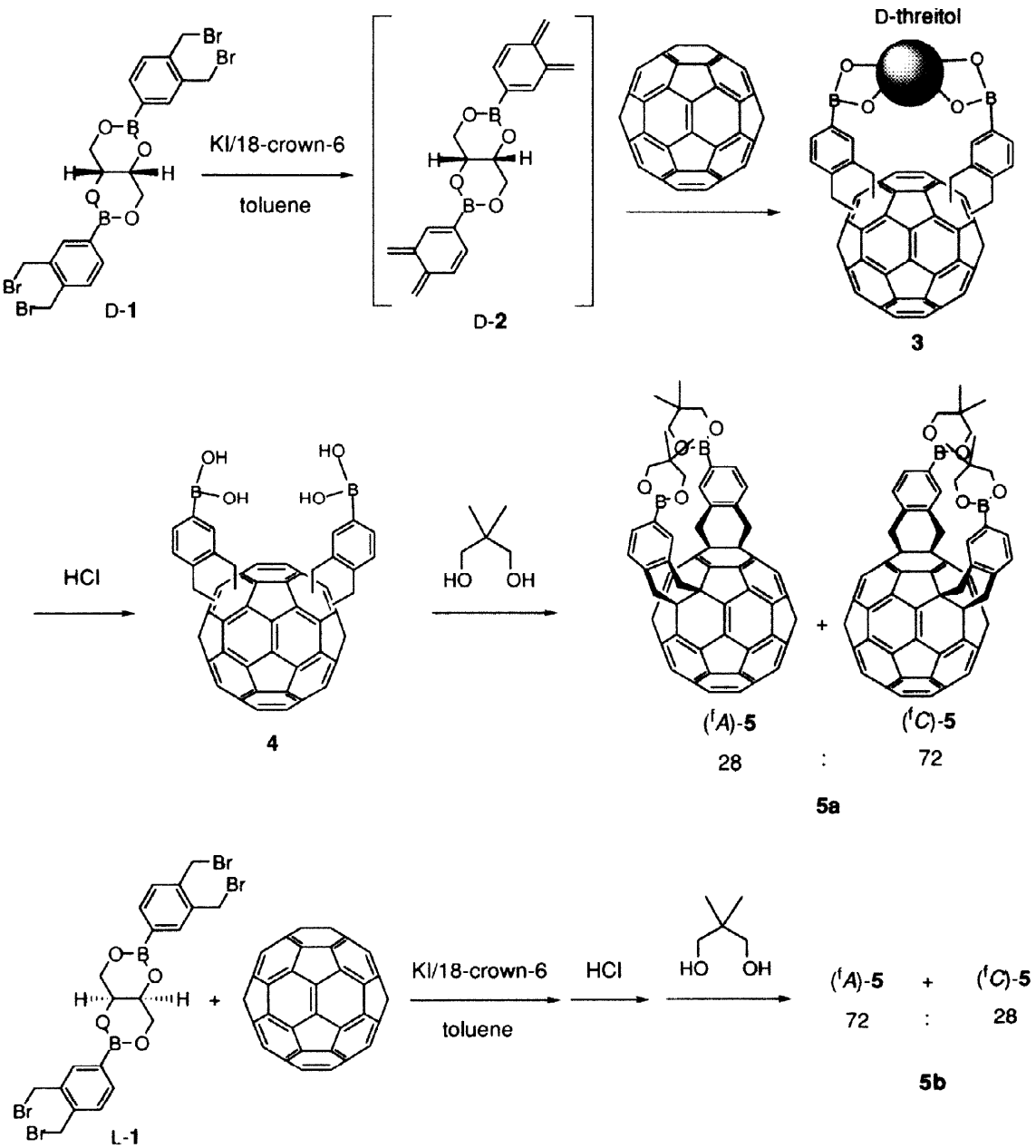
Scheme 1

In order to apply [60]fullerene to the creation of memory storage systems, we selected saccharides as templates as well as target molecules and boronic acids as functional groups. The principle idea is the regio- and chiroselective double [4+2] cycloaddition[3] between [60]fullerene and saccharide-[3,4-bis(bromomethyl)-phenylboronic acid] 1:2 complex and the subsequent removal and re-binding of a saccharide template based on the saccharide-boronic acid interaction[4] (Scheme 1). In the previous work, we reported that D-threitol- and L-threitol-boronic acid 1:2 complexes feature the regio- and chiroselective cycloaddition with [60]fullerene, affording the two optically active *cis*-3 bisadducts[5] with opposite chirality.[6] These chiroselective cycloaddition products have made it possible to test the D/L selective re-binding of the original template molecules. In this paper, we demonstrate that this new imprinting technique is really useful to create the memory storage systems for the saccharides used as the template molecules.

RESULTS AND DISCUSSION

As reported in our previous publications, the double addition between [60]fullerene and D-threitol-boronic acid 1:2 complex D-**1**, which was followed by treatment with HCl and by treatment with 2,2-dimethylpropane-1,3-diol, yielded C_2 symmetrical *cis*-3 bisadduct **5a** as a mixture of the two enantiomers (^fA)-**5**[7] and (^fC)-**5**[7] in the ratio of 28:72 (Scheme 2).[6] Thus, this mixture showed the optical activity because of the enantiomeric excess. The structure of **5a** was already identified by means of the spectroscopic methods, the chiral HPLC analysis, and the chemical conversion except for the absolute configuration.[6] Now, it is possible to assign the

absolute configuration of the major enantiomer of **5a** to be (^{f}C)-**5** based on the recent CD (circular dichroism) method reported by Harada and Diederich's group.[8] Similarly, the optically active mixture of **5b** with 44% excess of (^{f}A)-**5** was also obtained from L-threitol-boronic acid 1:2 complex L-**1** (Scheme 2).



Scheme 2

In order to rationalize the chiroselectivity of the double additions, the structure of the *o*-quinodimethane intermediate D-**2** generated from D-**1** was optimized by molecular orbital calculation[9] and the orientation between the two *o*-quinodimethane moieties in D-**2** was carefully checked. The initial structure of D-**2** was derived from the X-ray structure of D-**1**, which has the sofa form in the two six-membered boronate rings, by replacement of the bromomethyl groups to the sp^2 carbons.[10] As shown in Fig. 1, the optimized structure of D-**2** was very

similar to the X-ray structure of D-**1**. The top view drawing of D-**2** showed that the two *o*-quinodimethane moieties were situated in the left up and right down positions named as a *up-down* location. Here, we call the two enantiomeric location patterns in *cis*-3 bisadduct of [60]fullerene as *up-down* for the clockwise (^{f}C)-enantiomer and as *down-up* for the anticlockwise (^{f}A)-enantiomer, as shown in Fig. 2. By comparison of Fig. 1 with Fig. 2, it is clear that the *up-down* location of the two reactive groups in D-**2** is suitable for the *up-down* *cis*-3 location to give a (^{f}C)-enantiomer rather than for the *down-up* one to give a (^{f}A)-enantiomer. This speculation is commensurate with the result of the chiroselective double addition between [60]fullerene and D-**1**, which was controlled by the inherent chirality of the D-threitol template to give the (^{f}C)-enantiomer as a major product. Similarly, the preference of the (^{f}A)-enantiomer is rationalized in the L-threitol system.

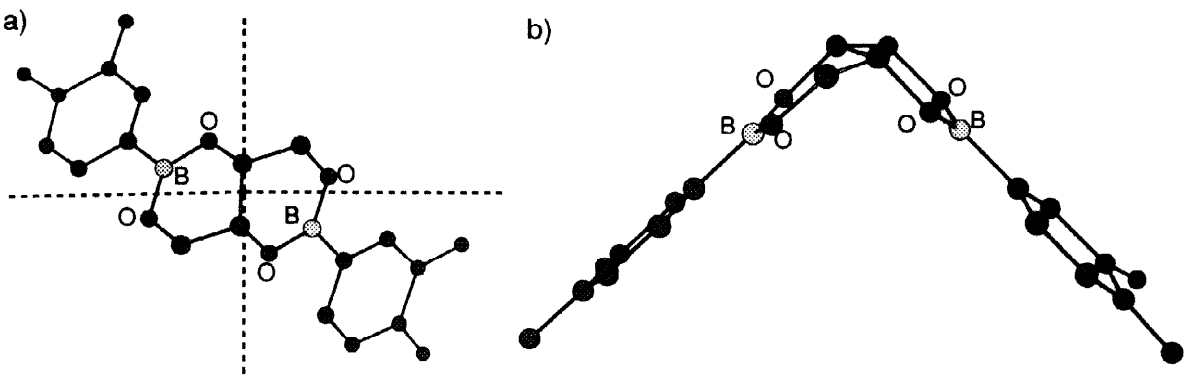


Fig. 1 Energy-minimized structure of the *o*-quinodimethane species D-**2** started from the X-ray structure of D-**1**: a) top view and b) side view.

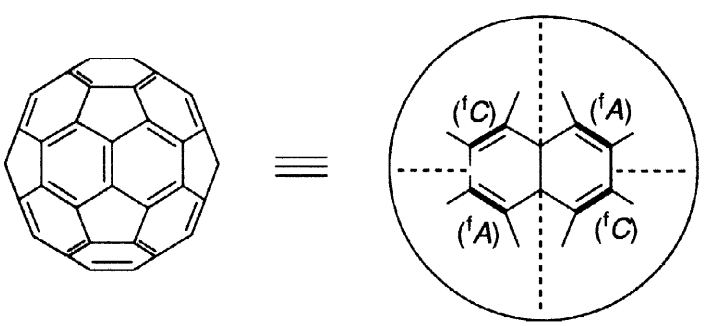
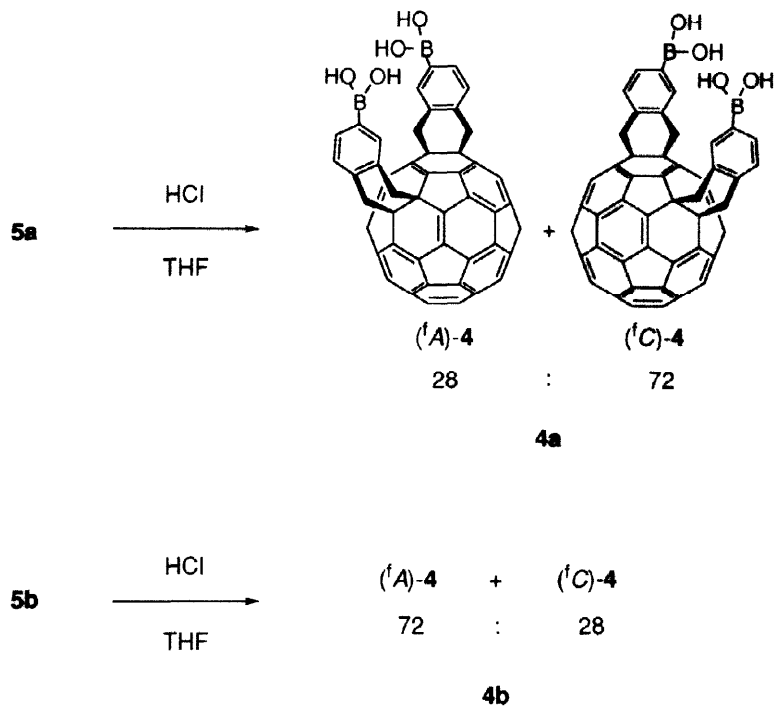


Fig. 2 Two enantiomeric *cis*-3 location patterns of [60]fullerene leading to a (^{f}C)-enantiomer (*up-down*) and to a (^{f}A)-enantiomer (*down-up*).

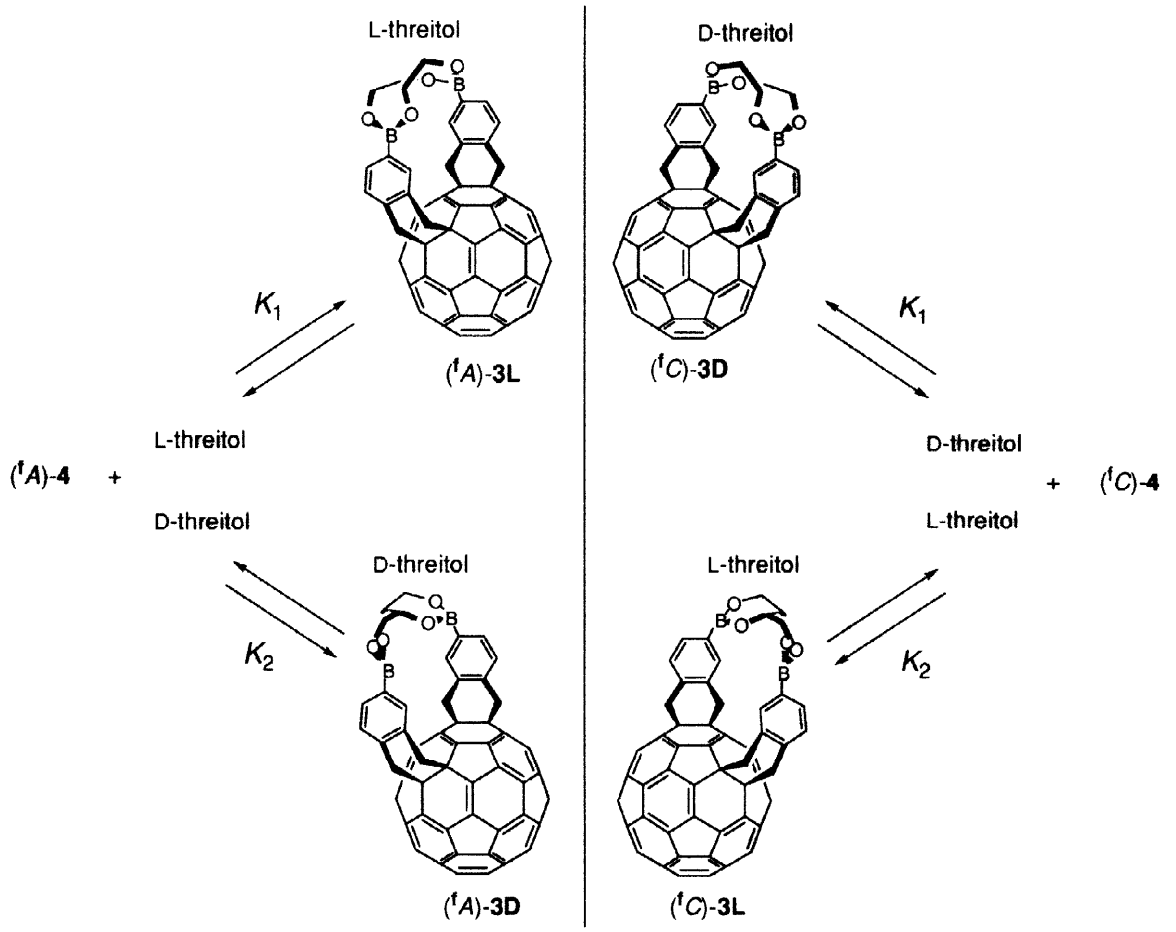
In order to evaluate the imprinting effect, the protective groups of **5a** and **5b** were removed by treatment with HCl to give the deprotected **4a** (composed of 28:72 ratio of (^{f}A)-**4** and (^{f}C)-**4**) and **4b** (composed of 72:28 ratio of (^{f}A)-**4** and (^{f}C)-**4**) in 57% and 45% yield, respectively (Scheme 3). The ^{1}H NMR, IR, and mass spectral data of **4a** and **4b** were strongly supportive of the removal of the protective groups from **5a** and **5b**.[11] The imprinting ability of **4a** and **4b** is estimated by a competitive complexation method.



Scheme 3

The competitive complexation of **4a** or **4b** with 5 equivalents of D-threitol and 5 equivalents of L-threitol was performed in toluene/methanol (15:2 v/v) at various temperatures. After the complexation was equilibrated, the reaction mixture was evaporated at the same temperature *in vacuo*. This treatment afforded the desired 1:1 cyclic complex **3** quantitatively. The structure of **3** was identified by means of ^{1}H NMR and IR spectroscopy and mass spectrometry.[11] In the ^{1}H NMR spectra of **3** obtained under these conditions, one could observe the two sets of peaks, which corresponded to a pair of isomers of **3** (see Experimental Section). The two doublet peaks at 7.80 ppm and 7.89 ppm for an aromatic proton of the two isomers of **3** were clearly distinguishable. Thus, the isomer ratio of **3** could be quantitatively estimated by the integration of the two aromatic proton peaks. The results are compiled in Table 1.

As shown in Scheme 4, the competitive complexation of (^{f}*A*)-**4** yields the two complexes, (^{f}*A*)-**3L** with L-threitol and (^{f}*A*)-**3D** with D-threitol. The former complex should be preferentially formed, since (^{f}*A*)-**4** was a major isomer in the reaction using L-threitol as a template. From (^{f}*C*)-**4**, on the other hand, the formation of the complex (^{f}*C*)-**3D** with D-threitol should be preferred to the complex (^{f}*C*)-**3L** with L-threitol. Since these two pathways in the competitive complexation are mirror images to each other, the two pairs of the preferential isomers (^{f}*A*)-**3L** and (^{f}*C*)-**3D** and the less preferential isomers (^{f}*A*)-**3D** and (^{f}*C*)-**3L** are diastereomeric to each other. This diastereomeric pair would correspond to the two isomers of **3** obtained from the competitive complexation of **4a** or **4b** with threitols. Although the two kinds of the competitive complexations occur in both **4a** and **4b**, the total imprinting effects for **4a** and **4b** are easily evaluated by the determination of diastereomeric ratio of **3**, which can be readily obtained from the ^{1}H NMR spectroscopic method.[12]



Scheme 4. Schematic explanation for the competitive complexation of (^{f}A)-**4** and (^{f}C)-**4** with threitols

In the competitive complexation of **4a** at 110 °C, one diastereoisomer was preferentially formed compared to another one in the ratio of 60:40 (run 1 in Table 1). In the case of **4b** under the same conditions, the same result was obtained (run 2 in Table 1). In order to check which is the major diastereoisomer, [(^{f}A)-**3L** + (^{f}C)-**3D**] or [(^{f}A)-**3D** + (^{f}C)-**3L**], the simple complexation of **4a** with D-threitol was performed. As expected, this complexation afforded a corresponding 72:28 mixture of (^{f}C)-**3D** and (^{f}A)-**3D** in the same ratio of the two enantiomers of **4a**. The ^{1}H NMR spectrum of the major complex (^{f}C)-**3D** coincided with that of the major diastereoisomer obtained from competitive complexation of **4a** or **4b**, indicating the preferential formation of a pair of (^{f}A)-**3L** and (^{f}C)-**3D** over that of (^{f}A)-**3D** and (^{f}C)-**3L**. The fraction of the preferential diastereoisomer [(^{f}A)-**3L** + (^{f}C)-**3D**] increased with lowering the reaction temperature (from 110 °C to −5 °C): the diastereomeric ratio was changed from 60:40 to 74:26 (runs 2–5 in Table 1). Under the optimum conditions (at −5 °C) one could obtain one diastereoisomer [(^{f}A)-**3L** + (^{f}C)-**3D**] in 48% de. The results of the competitive complexations strongly indicate that L-threitol-imprinted (^{f}A)-**4** and D-threitol-imprinted (^{f}C)-**4** diastereoselectively rebind with the original templates, L-threitol and D-threitol, leading (^{f}A)-**3L** and (^{f}C)-**3D**, respectively.

Table 1. Re-binding of **4a** or **4b** with D-threitol and L-threitol[a]

Run	Substrate	Temp. (°C)	Diastereomeric ratio (mol/mol) of **3** [(${}^{f}A$)-**3L** + (${}^{f}C$)-**3D** : (${}^{f}A$)-**3D** + (${}^{f}C$)-**3L**][b]
1	**4a**	110	60 : 40
2	**4b**	110	60 : 40
3	**4b**	85	62 : 38
4	**4b**	25	70 : 30
5	**4b**	-5	74 : 26

[a] **4a** or **4b** was competitively complexed with 5 equivalents of D-threitol and 5 equivalents of L-threitol in toluene/methanol (15:2 v/v).

[b] Checked by ^{1}H NMR in $CDCl_3$.

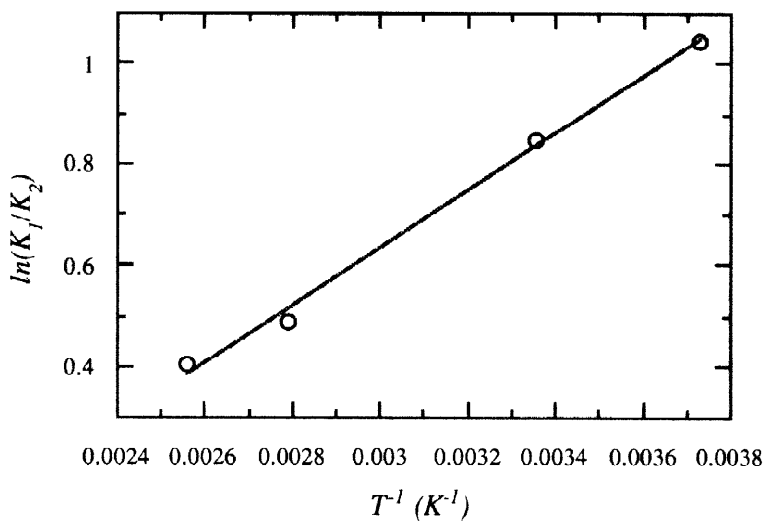


Fig. 3 van't Hoff plot for re-binding of **4b** with D-threitol and L-threitol; the correlation coefficient (R) is 0.998 where $\Delta(\Delta H^{\circ})$ is –4.71 kJ mol^{-1} and $\Delta(\Delta S^{\circ})$ is –8.84 J mol^{-1} K^{-1}.

The thermodynamic parameters between the formation of the two diastereoisomers [(${}^{f}A$)-**3L** + (${}^{f}C$)-**3D**] and [(${}^{f}A$)-**3D** + (${}^{f}C$)-**3L**] were estimated by a van't Hoff plot of the data for runs 2–5 in Table 1. The ratio of the equilibrium constants K_1 and K_2 in Scheme 4 was approximated to the molar ratio of [(${}^{f}A$)-**3L** + (${}^{f}C$)-**3D**] and [(${}^{f}A$)-**3D** + (${}^{f}C$)-**3L**]. In Fig. 3, the ln (K_1/K_2) values were plotted against T^{-1}, from which the $\Delta(\Delta H^{\circ})$ and $\Delta(\Delta S^{\circ})$ values were estimated to be –4.71 kJ mol^{-1} and –8.84 J mol^{-1} K^{-1}, respectively. This result implies that the formation of the preferential diastereoisomer [(${}^{f}A$)-**3L** + (${}^{f}C$)-**3D**] is thermodynamically more favored than that of the less preferential diastereoisomer [(${}^{f}A$)-**3D** + (${}^{f}C$)-**3L**]. Theoretically, the preferential diastereoisomer [(${}^{f}A$)-**3L** + (${}^{f}C$)-**3D**] could be obtained in 72% de at –78 °C (dry ice-acetone bath temperature), although such a low temperature reaction is experimentally very difficult. This thermodynamic preference of a pair of (${}^{f}A$)-**3L** and (${}^{f}C$)-**3D** over that of (${}^{f}A$)-**3D** and (${}^{f}C$)-**3L** is ascribed to the imprinting effect derived from

the chiroselective double additions between [60]fullerene and threitol-boronic acid 1:2 complexes. It is no doubt that this difference is controlled by the inherent chirality of threitol templates.

In this study, we have demonstrated that the chiroselectively saccharide-imprinted [60]fullerene-bisadducts diastereoselectively rebind with the original saccharides used as the template molecules. Although the selectivity in this system is not so high, it should be emphasized that the molecular imprinting technique is applied for the first time to a homogeneous solution system utilizing [60]fullerene as a soluble nano-size matrix. We can call this as a novel "homogeneous nano-scale imprinting system", because both the imprinting process and the estimation process can be performed in a reliable homogeneous system and the mechanistic details can be observed in the molecular level. We believe that the present study is more generally applicable to the creation of various memory storage systems for the various template molecules.

EXPERIMENTAL

All melting points are uncorrected. IR spectra were recorded on a SHIMAZU FT-IR 8100M and measured as KBr pellets. ^{1}H NMR spectra were determined in $CDCl_3$ and 1,4-dioxane-d_8 with a BRUKER ARX300. Mass spectra (negative SIMS) were measured on a HITACHI M-2500 Mass Spectrometer. D-Threitol and L-threitol were purchased from SIGMA Co., Ltd.

65,73-Bis(dihydroxybora)-1,2:16,17-bis(methano[1,2]benzenomethano)[60]fullerene (4).

To a solution of **5a** (40 mg, 0.0347 mmol) in THF (3.5 mL) was added 1.2 mol dm^{-3} hydrochloric acid (1.8 mL) at 0 °C under a nitrogen atmosphere. After the mixture was stirred for 17 h at room temperature, it was washed with brine, dried over anhydrous magnesium sulfate, and evaporated *in vacuo* to dryness. The residue was washed with dichloromethane to give **4a** in 57% yield (20 mg, 0.0197 mmol): mp >450 °C; IR (KBr) ν 1412 (ν_{BC}), 1362, 1318, (ν_{BO}), 687, 523 cm^{-1}; ^{1}H NMR (1,4-dioxane-d_8, 25 °C) δ 3.39, 4.03, 4.08, 4.51 (d, J = 13.5 Hz, each 2 H, CH_2), 7.15 (s, 2 H, ArH), 7.45 (d, J = 7.4 Hz, 2 H, ArH), 7.57 (s, D_2O-exchange, 4 H, OH), 8.04 (d, J = 7.4 Hz, 2 H, ArH); MS (negative SIMS, NBA) m/z 1015 [(M–1)$^-$].

Similarly, **4b** was obtained from **5b** (35 mg, 0.0304 mmol), THF (3 mL), and 1.2 mol dm^{-3} hydrochloric acid (1.5 mL) in 45% yield (14 mg, 0.0138 mmol). The spectral data of **4b** coincided with those of **4a**.

General Procedure for Competitive Complexation of 4a or 4b with D-Threitol and L-Threitol.

To a suspension of **4a** or **4b** (3.1 mg, 0.003 mmol) in dry toluene (1.5 mL) was added 0.15 mol dm^{-3} methanol solution of 1:1 mixture of D-threitol and L-threitol (0.2 mL, 0.015 mmol for each threitol) at room temperature under a nitrogen atmosphere. Immediately, the brown suspension was changed to the clear brown solution. Then, the mixture was stirred at various temperatures for 14 h and evaporated at the same temperature *in vacuo* to dryness. The residue was suspended in dry toluene (2 mL) and filtered off to remove the unreacted threitols. The filtrate was evaporated *in vacuo* quantitatively to give **3** as an isomeric mixture. When the complexation of **4a** or **4b** with D-threitol and L-threitol was finished within 3-5 h, **3** could not be obtained quantitatively and the starting material **4a** or **4b** was recovered. Normally the complexation was equilibrated over 10 h under the several conditions.

3: brown solid (dichloromethane/hexane); mp >450 °C; IR (KBr) ν 2919, 2840, 1418 (ν_{BC}), 1358, 1323, (ν_{BO}), 903, 727, 525 cm^{-1}; ^{1}H NMR ($CDCl_3$, 25 °C) for major diastereoisomer [(^{f}*A*)-**3L** + (^{f}*C*)-**3D**] δ

3.59, 3.98 (d, J = 14.1 Hz, each 2 H, CH_2), 4.05 (d, J = 13.8 Hz, 2 H, CH_2), 4.36–4.77 (m, 8 H, CH_2 and threitol-CH), 7.38–7.47 (m, 4 H, ArH), 7.80 (d, J = 7.3 Hz, 2 H, ArH), for minor diastereoisomer [(^fA)-**3D** + (^fC)-**3L**] δ 3.54, 3.98 (d, J = 14.1 Hz, each 2 H, CH_2), 4.06 (d, J = 13.8 Hz, 2 H, CH_2), 4.36–4.77 (m, 8 H, CH_2 and threitol-CH), 7.38–7.47 (m, 4 H, ArH), 7.89 (d, J = 7.3 Hz, 2 H, ArH); MS (negative SIMS, NBA) m/z (%) 1066 (M^-).

Computational Method.

Energy-minimization of D-**2** was carried out by MOPAC AM1 using CS Chemoffice Chem 3D (Cambridge Soft Corporation) on a Macintosh computer.[9]

van't Hoff Plot for Competitive Complexation of 4b with D-Threitol and L-Threitol.

In the competitive complexation of **4b** with D-threitol and L-threitol (runs 2–5 in Table 1), the thermodynamic parameters $\Delta(\Delta H^\circ)$ and $\Delta(\Delta S^\circ)$ were obtained from a van't Hoff plot according to $\ln(K_1/K_2) = \Delta(\Delta H^\circ)\cdot R^{-1}\cdot T^{-1} + \Delta(\Delta S^\circ)\cdot T^{-1}$: K_1/K_2; which was approximated to the diastereomeric ratio of [(^fA)-**3L** + (^fC)-**3D**] and [(^fA)-**3D** + (^fC)-**3L**], $\Delta(\Delta H^\circ)$; difference from ΔH° of complex [(^fA)-**3L** + (^fC)-**3D**] to ΔH° of complex [(^fA)-**3D** + (^fC)-**3L**], $\Delta(\Delta S^\circ)$; difference from ΔS° of [(^fA)-**3L** and (^fC)-**3D**] to ΔS° of [(^fA)-**3D** + (^fC)-**3L**], T; complexation temperature. The $\ln(K_1/K_2)$ values were plotted against T^{-1}, from which the $\Delta(\Delta H^\circ)$ and $\Delta(\Delta S^\circ)$ values were estimated to be –4.71 kJ mol^{-1} and –8.84 J mol^{-1} K^{-1}, respectively.

REFERENCES AND NOTES

1. For reviews see: a) Wulff, G. *Angew. Chem. Int. Ed. Engl.*, **1995**, *34*, 1812–1832. b) Kriz, D.; Ramström, O.; Mosbach, K. *Anal. Chem.*, **1997**, *69*, 345A–349A. c) Lindsey, L. S. *New. J. Chem.*, **1991**, *15*, 153–180 (section III-D-3, pp 167–168).
2. a) Wulff, G.; Schauhoff, S. *J. Org. Chem.*, **1991**, *56*, 395–400. b) Vlatakis, G.; Andersson, L. I.; Müller, R.; Mosbach, K. *Nature*, **1993**, *361*, 645–647. c) Mathew-Krotz, J.; Shea, K. J. *J. Am. Chem. Soc.*, **1996**, *118*, 8154–8155. d) Spivak, D.; Gilmore, M. A.; Shea, K. J. *J. Am. Chem. Soc.*, **1997**, *119*, 4388–4393. e) Yu, C.; Mosbach, K. *J. Org. Chem.*, **1997**, *62*, 4057–4064.
3. a) Belik, P.; Gügel, A.; Spickermann, J.; Müllen, K. *Angew. Chem. Int. Ed. Engl.*, **1993**, *32*, 78–80. b) Taki, M.; Sugita, S.; Nakamura, Y.; Kasashima, E.; Yashima, E.; Okamoto, Y.; Nishimura, J. *J. Am. Chem. Soc.*, **1997**, *119*, 926–932.
4. For reviews see: a) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Angew. Chem. Int. Ed. Engl.*, **1996**, *35*, 1910–1922. b) James, T. D.; Linnane, P.; Shinkai, S. *Chem. Commun.*, **1996**, 281–288.
5. Naming of [60]fullerene-bisadducts: Hirsch, A.; Lamparth, I.; Karfunkel, H. R. *Angew. Chem. Int. Ed. Engl.*, **1994**, *33*, 437–438.
6. a) Ishi-i, T.; Nakashima, K.; Shinkai, S. *Chem. Commun.*, **1998**, 1047–1048. b) Ishi-i, T.; Nakashima, K.; Shinkai, S.; Ikeda, A. *J. Org. Chem.*, in press.
7. For (^fC) and (^fA), the three signs, f, C, and A mean fullerene, clockwise, and anticlockwise, respectively. For naming of chiral fullerene derivatives see: Thilgen, C.; Herrmann, A.; Diederich, F. *Helv. Chim. Acta*, **1997**, *80*, 183–199.

8. Recently, Harada and Diederich's group assigned the absolute configurations of inherently chiral fullerene derivatives based on the comparison between the theoretical and experimental CD spectra: Goto, H.; Harada, N.; Crassous, J.; Diederich, F. *J. Chem. Soc. Perkin Trans. 2*, **1998**, 1719–1723. The calculation of the theoretical CD spectra was performed by a π-electron SCF-CI-DV MO (self consistent field-configuration interaction-dipole velocity molecular orbital) method. For example, a chiral *cis*-3 isomer of [60]fullerene-bisadduct with the plus sign of a band around 490 nm can be identified to be (^{f}C)-configuration. The CD spectrum of **5a** obtained here was very similar to this typical (^{f}C)-pattern, showing that the major enantiomer of **5a** is (^{f}C)-**5** but not (^{f}A)-**5**. Similarly, the major isomer of **5b** was identified to be (^{f}A)-**5**. The CD spectra of **5a** and **5b** were already reported in reference 6b.
9. a) James, T. D.; Harada, T.; Shinkai, S. *J. Chem. Soc. Chem. Commun.*, **1993**, 857–860. b) James, T. D.; Kawabata, H.; Ludwig, R.; Murata, K.; Shinkai, S. *Tetrahedron*, **1995**, *51*, 555–566. c) Takeuchi, M.; Chin, Y.; Imada, T.; Shinkai, S. *Chem. Commun.*, **1996**, 1867–1868.
10. Ishi-i, T.; Nakashima, K.; Shinkai, S.; Araki, K. *Tetrahedron*, **1998**, *54*, 8679–8686.
11. Although the elemental analyses of **3** and **4** were also measured, the observed values did not coincided with the calculated values because of the contamination of a trace amount of solvents. Further, for **4** the existence of a few different structures such as free boronic acid, boronic acid anhydride, and cyclic trimer 2,4,6-*o*-triarylboroxin may cause the disagreement in elemental analysis. The high resolution mass spectrometry of **3** and **4** could not be performed because of the very low intensities of the parent ion peaks of **3** and **4**.
12. Firstly, we attempted to evaluate the imprinting effect by an enantiomer labelled guest method using mass spectrometry: Sawada, M.; Takai, Y.; Yamada, H.; Hirayama, S.; Kaneda, T.; Tanaka, T.; Kamada, K.; Mizooku, T.; Takeuchi, S.; Ueno, K.; Hirose, K.; Tobe, Y.; Naemura, K. *J. Am. Chem. Soc.*, **1995**, *117*, 7726–7736. In the mass spectra (negative SIMS, NBA) of **3**, which was obtained from the competitive complexation of **4a** with D-threitol and *deuterium labelled* 1,1,4,4-^{2}H4-L-threitol, the intensity of the parent ion peak (m/z, 1066) for D-threitol complex [(^{f}C)-**3D** + (^{f}A)-**3D**] was always higher than that of the parent ion peak (m/z, 1070) for 1,1,4,4-^{2}H4-L-threitol complex [(^{f}A)-**3L**-*d*4 + (^{f}C)-**3L**-*d*4] during 30-40 scans, as expected. However, this method was applicable only to qualitative analysis because of the lack of the scan stability. We later found that the imprinting effect can be quantitatively evaluated by using a ^{1}H NMR spectroscopic method.