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A study of the strength of a template molecule - a functional monomer interaction that affects the performance of molecularly imprinted polymers and its application to chiral amplification

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Summary

A novel type of molecularly imprinted polymer (MIP), *N*-benzoyl-(*S*)-valine anilide-imprinted polymer (**IP-2**), was prepared using hydrogen-bonding interactions as a main force in the pre-polymerization step. The performance of the **IP-2** was evaluated via batch procedure and compared with a (*S*)-valine anilide-imprinted polymer (**IP-1**) that was prepared using an ionic interaction that is stronger than hydrogen bonding. Although both polymers showed a preferential adsorbability for (*S*)-amino acid derivatives, different performances were observed in terms of adsorbability and enantioselectivity. In addition, the **IP-2** was able to recognize the enantiomer of a valine-derived chiral catalyst. This phenomenon was applied to a chiral amplification reaction, and a highly selective asymmetric Mannich-type amination was achieved using the combination of a racemic catalyst and a MIP.

Keyword: molecularly imprinted polymer, chiral recognition, (*S*)-benzoyl amino acid anilide, asymmetric amplification

Introduction

Molecularly imprinted polymers (MIPs)[1-3] have been energetically pursued in the field of molecular recognition, and various types of applications such as a stationary phase of HPLC,[4,5] solid-state extraction,[6] and chemical sensors[7,8] have been reported due to qualities such as synthetic simplicity, stability, and a high level of recognition selectivity.

There are several methods for the preparation of imprinted polymers, including acid-base interaction, simple hydrogen bonding, dipole-dipole interaction, and hydrophobic interaction at the pre-polymerization step. Among these, the acid-base interaction[4,5,9] and/or hydrogen bonding[10,11] are mainly used due to the effectiveness of bonding strength in regards to the tailor-made construction of three-dimentional binding sites.

Therefore, MIPs prepared using an acid-base interaction appear to show a higher level of performance than those without such an ionic interaction. However, unexpectedly, only a few reports[12] described the comparison of the different performances of MIPs between the presence of acid-base interaction and the absence of such a strong interaction. Therefore, it would be desirable to establish a new methodology, which would enable a convenient and precise comparison of the performance of MIPs that could lead to the development of a new type of MIP.

We previously reported[13] the evaluation of *basic* (*S*)-amino acid anilide (such as (*S*)-**1**)-imprinted MIPs that were prepared using an acid-base interaction by batch procedure that allowed independent observation of adsorbability and selectivity, which were not precisely measured by a HPLC column system.

In this paper we describe the evaluation of the performance of *neutral* (*S*)-*N*-benzoyl amino acid anilide (such as (*S*)-**5**)-imprinted MIPs, which were prepared in the presence of a weaker hydrogen-bonding interaction in the pre-polymerization step, by batch procedure. We also report a MIP with a (*S*)-*N*-benzoyl valine anilide cavity and its application to chiral amplification.

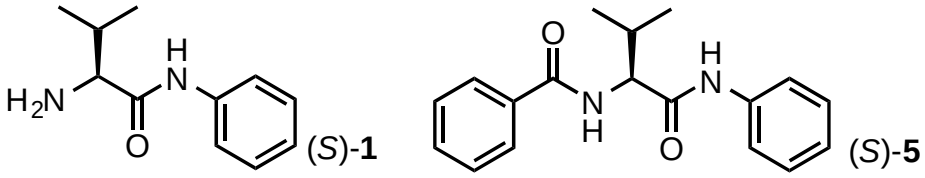


Fig. 1. Structures of (*S*)-**1** and (*S*)-**5**.

Experimental

General Ethylene glycol dimethacrylate (EGDM), methacrylic acid (MAA) and chloroform were freshly distilled prior to use. (*RS*)-alanine, (*S*)- and (*RS*)-valine, (*RS*)-phenylalanine, (*RS*)-*tert*-leucine, methacryloyl chloride, 2,2′-azobis(isobutyronitrile) (AIBN), 2-aminoethanol, *p*-toluenesulfonyl chloride, cyclohexane (*c*-hex), diethylcyanophosphonate (DEPC), *o*-anisidine, *o*-anisic acid, $La(NO_3)_3 \cdot 6H_2O$ and H-D-Val-O^{t}Bu were purchased and used without further purification. *n*-hexane (*n*-hex), 2-propanol, ethanol (EtOH), and acetonitrile were all HPLC grade and used without further purification. Tetrahydrofuran (THF; dehydrated) and dichloromethane (CH_2Cl_2; dehydrated) were purchased (Kanto Chemical, Co., Inc.), stored under an argon atmosphere and used without further purification. Diethyl ether (Et_2O), ethyl acetate (EtOAc), *N*,*N*'-dimethylformamide (DMF) and methanol (MeOH) were purified by distillation.

The preparation of (*S*)-valine anilide-imprinted polymers (**IP-1**), using an acid-base interaction, and the adsorbability of various types of amino acid anilides onto **IP-1** have been reported.[13)]

Synthesis

(*S*)-*N*-benzoyl-valine anilide ((*S*)-5); general procedure for the preparation of *N*-benzoyl amino acids: (*S*)-valine anilide ((*S*)-**1**) (1.34 g, 7.00 mmol) and benzoic acid (0.900 g, 7.35 mmol) were dissolved in DMF (41.7 mL). To the mixed solution was added Et_3N (1.10 mL, 7.89 mmol) and DEPC (1.10 mL, 7.35 mmol), and the solution was stirred at 0 ^oC for 45 min. The resultant mixture was diluted with EtOAc (300 mL), and the organic layer was washed with satd. $NaHCO_3$ aq. (50 mL x 3) and satd. NaCl aq. (50 mL x 3) and dried over anhyd. Na_2SO_4. After concentration of the organic layer *in vacuo.*, the residue was

purified by recrystallization (hexane-CH_2Cl_2) to give (*S*)-**5** (1.60 g, 5.38 mmol) as a colorless solid: m.p. 216-218 ^oC; $[\alpha]_D^{22}$ -46.9 (*c* 0.52, $CHCl_3$); ^{1}H-NMR (300 MHz/$CDCl_3$) δ: 9.54 (1H, br s), 7.82-7.80 (2H, m), 7.51-7.45 (4H, m), 7.36-7.33 (2H, m), 7.05-7.02 (1H, m), 4.98 (1H, t, *J* = 8.4Hz), 2.41-2.36 (1H, m), 1.16 (3H, d, *J* = 7.0 Hz), 1.14 (3H, d, *J* = 7.0 Hz); ^{13}C-NMR (75 MHz/$CDCl_3$) δ: 170.5, 168.1, 137.8, 133.9, 131.7, 128.7, 128.5, 127.3, 124.2, 120.3, 60.0, 31.7, 19.3, 18.9; IR (KBr) 3278, 1668, 1633, 1603, 1531, 1446 cm^{-1}; HRMS (FAB) *m/z* calcd. for $C_{18}H_{21}N_2O_2$ $[M+H]^+$ 297.1603, found 297.1586.

Racemic *N*-benzoyl amino acids, (*RS*)-**6**, **7** and **8** were synthesized by the same procedure as (*S*)-**5** from corresponding amino acid derivatives. Then, (*RS*)-**6** and (*RS*)-**8** were purified by recrystallization from hexane-CH_2Cl_2, and (*RS*)-**7** was purified by recrystallization from EtOAc.

(*RS*)-*N*-benzoyl-alanine anilide ((*RS*)-6): Colorless solid; m.p. 169-171 ^oC; ^{1}H-NMR (300 MHz/$CDCl_3$) δ: 9.42 (1H, br s), 7.87-7.84 (2H, m), 7.60 (2H, d, *J* = 7.7 Hz), 7.52 (1H, dd, *J* = 9.5, 7.7 Hz), 7.44-7.40 (3H, m), 7.31-7.25 (2H, m), 7.11-7.06 (1H, m), 5.15-5.05 (1H, m), 1.58 (3H, d, *J* = 7.0 Hz); ^{13}C-NMR (75 MHz/$CDCl_3$) δ: 170.9, 167.7, 138.0, 133.5, 132.0, 128.9, 128.6, 127.2, 124.3, 120.0, 50.1, 18.5; IR (KBr) 3297, 1686, 1633, 1606, 1548, 1489, 1442 cm^{-1}; HRMS (FAB) *m/z* calcd for $C_{16}H_{17}N_2O_2$ $[M+H]^+$ 269.1290, found 269.1284.

(*RS*)-*N*-benzoyl-phenylalanine anilide ((*RS*)-7): Colorless solid; m.p. 236-239 ^oC; ^{1}H-NMR (300 MHz/DMSO-d_6) δ: 10.20 (1H, br s), 8.72 (1H, d, *J* = 8.1 Hz), 7.83 (2H, d, *J* = 6.6 Hz), 7.62 (2H, d, *J* = 8.1 Hz), 7.54-7.40 (5H, m), 7.34-7.25 (4H, m), 7.17 (1H, dd, *J* = 7.7, 6.6 Hz), 7.08-7.03 (1H, m), 4.87-4.82 (1H, m), 3.17-3.11 (2H, m); ^{13}C-NMR (75 MHz/DMSO-*d6*) δ: 170.4, 166.5, 138.9, 138.1, 133.9, 131.3, 129.2, 128.7, 128.1, 128.1, 128.0, 127.4, 126.3, 123.3, 119.3, 55.8, 37.2; IR (KBr) 3292, 3268, 1676, 1635, 1551, 1535, 1446 cm^{-1}; HRMS (FAB) *m/z* calcd for $C_{22}H_{21}N_2O_2$ $[M+H]^+$ 345.1603, found 345.1583.

(*RS*)-*N*-benzoyl-*tert*-leucine anilide ((*RS*)-8): Colorless solid; m.p. 226-228 ^oC; ^{1}H-NMR

(300 MHz/$CDCl_3$) δ: 9.50 (1H, br s), 7.87-7.84 (2H, m), 7.57-7.52 (3H, m), 7.48-7.43 (2H, m), 7.27-7.20 (3H, m), 7.09-7.04 (1H, m), 5.17 (1H, d, *J* = 9.2 Hz), 1.14 (9H, s); ^{13}C-NMR (75 MHz/$CDCl_3$) δ: 169.4, 167.5, 137.9, 134.1, 131.9, 128.8, 128.72, 128.71, 127.1, 124.2, 120.0, 61.2, 35.9, 26.7; IR (KBr) 3305, 3267, 2962, 1678, 1627, 1554, 1539, 1490, 1446 cm^{-1}; HRMS (FAB) *m/z* calcd for $C_{19}H_{23}N_2O_2$ $[M+H]^+$ 311.1760, found 311.1741.

(*RS*)-*N*-(2-hydroxyphenyl)-2-(2-hydroxybenzoylamino)-3-methylbutylamide ((*RS*)-9): (*RS*)-**9** was synthesized by the same procedure as previously reported.[14] Colorless solid; m.p. 195-197 °C; ^{1}H-NMR (300 MHz/DMSO-d_6) δ: 11.81 (1H, s), 9.76 (1H, s), 9.41 (1H, s), 8.87 (1H, d, *J* = 8.2 Hz), 7.98 (1H, d, *J* = 7.9 Hz), 7.75 (1H, d, *J* = 7.9 Hz), 7.42-7.38 (1H, m), 6.95-6.85 (4H, m), 6.79-6.74 (1H, m), 4.69 (1H, dd, *J* = 7.7, 7.3 Hz), 2.28-2.21 (1H, m), 1.00 (3H, d, *J* = 5.7 Hz), 0.98 (3H, d, *J* = 5.9 Hz).

(*S*)-*N*-(2-methoxyphenyl)-2-(2-methoxybenzoylamino)-3-methylbutyramide ((*S*)-10): To a mixture of (*S*)-*N*-Boc-Val-OH (2.50 g, 11.5 mmol) and Et_3N (1.60 mL, 11.5 mmol) in THF (30 mL) was added ethyl chloroformate (1.10 mL, 11.5 mmol) in THF (13 mL) dropwise over 15 minutes at -15 °C. After stirring for 15 minutes, *o*-anisidine (1.36 mL, 12.1 mmol) was subsequently added and stirred at r.t. for 15 hours. The resultant mixture was diluted with EtOAc (300 mL), then washed with 10% $KHSO_4$ aq. (50 mL x 3), satd. $NaHCO_3$ aq. (50 mL x 3), satd. NaCl aq. (50 mL x 3) and dried over anhyd. Na_2SO_4. After concentration *in vacuo*, the residual solid was washed with a mixture of hexane- Et_2O. To a solution of the washed solid (2.38 g) in CH_2Cl_2 was added Amberlyst 15 (19.6 g) with stirring at r.t. for 15 hours. The resultant mixture was filtered, and the resin was washed with hexane, MeOH and THF. The resin was poured into NH_3/^{i}PrOH (197 mL) and stirred at r.t. for 40 minutes. The resin was filtered off, and the filtrate was concentrated *in vacuo.* The residue was dissolved in DMF (35 mL) and *o*-anisic acid (0.930 g, 6.10 mmol); Et_3N (0.85 mL, 6.10 mmol) and DEPC (0.870 mL, 5.80 mmol) were sequentially added at 0 °C followed by stirring at 0 °C for 30 min. The resultant mixture was diluted with EtOAc (300 mL), washed (satd. $NaHCO_3$ aq. (50 mL x 3) and satd. NaCl aq. (50 mL x 3)) and dried over anhyd. Na_2SO_4. After concentration

in vacuo, the residue was purified by silica gel column chromatography (CH_2Cl_2 to CH_2Cl_2/EtOAc (97.5: 7.5)) to give (*S*)-**10** as a colorless solid (1.60 g, 4.49 mmol); m.p. 108-110 °C; $[\alpha]_D^{22}$ +39.9 (*c* 0.70, $CHCl_3$); ^{1}H-NMR (300 MHz/$CDCl_3$) δ: 8.51 (1H, d, *J* = 8.4 Hz), 8.38 (1H, br s), 8.35 (1H, dd, *J* = 7.9, 1.6 Hz), 8.21 (1H, dd, *J* = 7.7, 1.8 Hz), 7.50-7.44 (1H, m), 7.11-6.83 (5H, m), 4.71 (1H, dd, *J* = 8.4, 5.9 Hz), 4.01 (3H, s), 3.80 (3H, s), 2.44-2.40 (1H, m), 1.09 (3H, d, *J* = 6.8 Hz), 1.07 (3H, d, *J* = 6.8 Hz); ^{13}C-NMR (75 MHz/$CDCl_3$) δ: 169.4, 165.4, 157.6, 148.1, 133.0, 132.3, 127.3, 123.9, 121.2, 121.0, 120.9, 119.9, 111.4, 110.0, 59.9, 56.1, 55.7, 30.8, 19.4, 18.0; IR (KBr) 3305, 2953, 1668, 1630, 1535, 1493, 1461, 1432 cm^{-1}; HRMS (FAB) *m/z* calcd for $C_{20}H_{25}N_2O_4$ $[M+H]^+$ 357.1814, found 357.1812.

Synthesis of (*S*)-*N*-phenyl-2-(2-methoxybenzoylamino)-3-methylbutyramide ((*S*)-11): (*S*)-valine anilide ((*S*)-**11**) (200 mg, 1.04 mmol) and *o*-anisic acid (166 mg, 1.09 mmol) were dissolved in DMF (6.20 mL). Et_3N (0.150 mL, 1.10 mmol) and DEPC (0.160 mL, 1.00 mmol) were added sequentially to the solution at 0 °C, and the mixture was stirred at 0 °C for 45 min. The resultant mixture was diluted with EtOAc (150 mL) and washed with satd.$NaHCO_3$ aq. (50 mLx3), satd. NaCl aq. (50 mLx3) and dried over anhyd. Na_2SO_4. After the organic layer was concentrated *in vacuo,* the residue was purified by silica gel column chromatography (CH_2Cl_2 to CH_2Cl_2/EtOAc (87.5: 12.5)) to give a colorless solid; m.p. 103-105 °C; $[\alpha]_D^{22}$ -30.6 (*c* 0.54, $CHCl_3$); ^{1}H-NMR (300 MHz/$CDCl_3$) δ: 8.82 (1H, br s), 8.54 (1H, d, *J* = 8.2 Hz), 8.20 (1H, dd, *J* = 7.9, 1.5 Hz), 7.54-7.46 (3H, m), 7.27-7.24 (2H, m), 7.08-7.03 (3H, m), 4.74 (1H, dd, *J* = 8.2, 6.9 Hz), 4.02 (3H, s), 2.50-2.38 (1H, m), 1.12 (3H, d, *J* = 6.8 Hz), 1.10 (3H, d, *J* = 6.8 Hz); ^{13}C-NMR (75 MHz/$CDCl_3$) δ: 170.0, 165.8, 138.0, 133.2, 132.2, 128.7, 124.0, 121.2, 121.0, 120.1, 111.5, 59.8, 56.1, 30.9, 19.5, 18.4; IR (KBr) 3284, 2958, 1635, 1601, 1535, 1489, 1444 cm^{-1}; HRMS (FAB) *m/z* calcd for $C_{19}H_{23}N_2O_3$ $[M+H]^+$ 327.1709, found 327.1719.

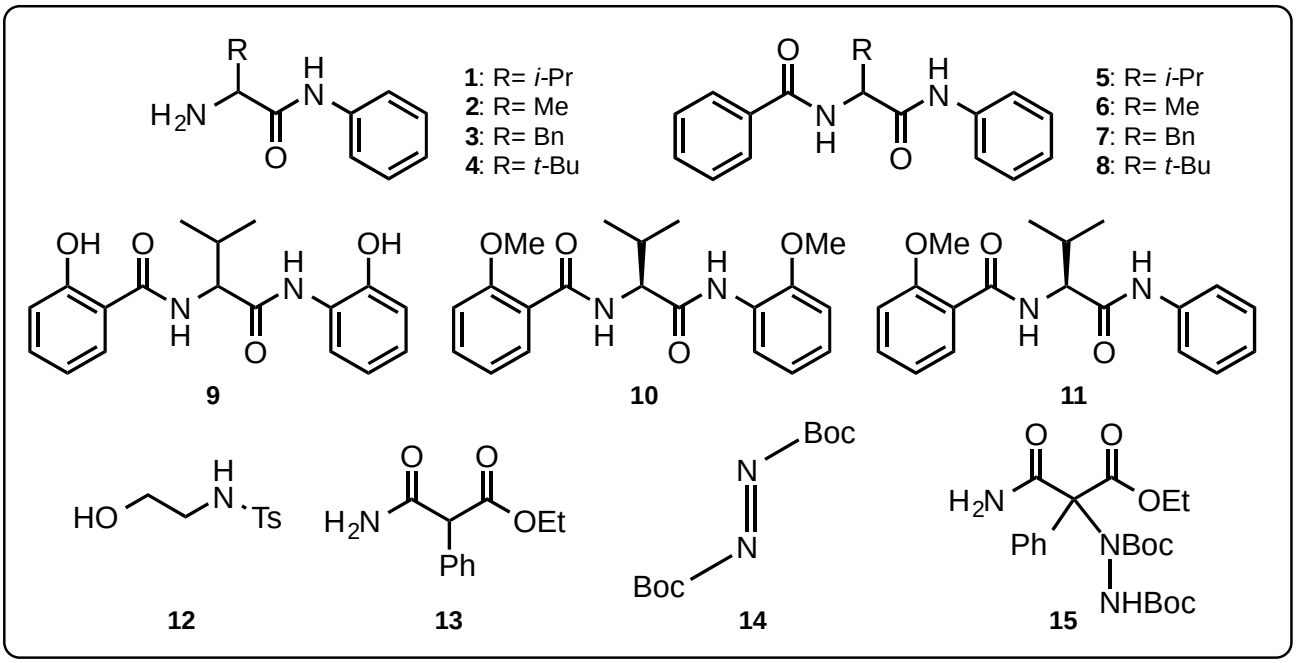


Fig. 2. Structures of the compounds.

General Procedure for the Preparation of Polymers:

Preparation of a Molecular-Imprinted Polymer (MIP) (Table 1)**:** We prepared several types of polymers as follows. A *N*-benzoyl-(*S*)-valine anilide ((*S*)-**5)**-imprinted MIP (**IP-2**) was prepared by the polymerization of a mixture of (*S*)-**5**, ethylene glycol dimethacrylate (EGDM), methacrylic acid (MAA), and AIBN in chloroform under irradiation followed by an appropriate amount of grinding and washing. In a similar manner, **IP-3** and **IP-4** were prepared by following the **IP-2** procedure. In a glass vial, (*S*)-**5** (889 mg, 3.00 mmol) was dissolved in chloroform (12.0 mL). To this solution were added MAA (1.00 mL, 12.0 mmol), EGDM (9.00 mL, 48.0 mmol), and AIBN (99.6 mg, 0.610 mmol). After sufficient mixing, the solution was separated into four borosilicate screw-capped test tubes, and each test tube was flushed with argon gas followed by irradiation with a 100 W high-pressure mercury vapor lamp (5°C, 24 h) to form a polymer. Bulk polymers were ground with a mill (OSAKA chemical, Wonder Blender, WB-1) and a ball mill (Nitto kagaku, ANZ-50S). These were then sieved through a 500 μm mesh filter, and particles of <500 μm were collected. The polymer was washed (by soxhlet extractor (MeOH/AcOH (7: 3)), 20 h), filtered (Kiriyama funnel (filter paper: No. 5B)), immersed in THF, and refluxed for 5 hours. The polymer was

collected by filtration (Kiriyama funnel (filter paper: No. 5B)), washed again (CH_2Cl_2), and dried *in vacuo*.

The preparation of a (*S*)-valine anilide ((*S*)-**1**)-imprinted MIP (**IP-1**) has been reported previously.[13)]

Table 1. Composition of the MIPs.

Entry	Polymer	Template	Template (mmol)	MAA (mmol)	EGDM (mmol)	$CHCl_3$ (mL)
1	**IP-1**[a)]	(*S*)-**1**	1	4	16	4
2	**IP-2**	(*S*)-**5**	3	12	48	12
3	**IP-3**	**10**	0.25	1	4	1
4	**IP-4**	**11**	0.25	1	4	1

a) See ref. 13.

General Procedure for Evaluating the Ability of a MIP Using the "Batch Procedure": In a glass vial, (*RS*)-**5** (2.60 μmol) was dissolved in a mixture of CH_2Cl_2 and *n*-hexane (10 mL; CH_2Cl_2/ hexane (1: 1)). This concentration was defined as $[\mathbf{5}]_0$, which was an average of $[\mathbf{5}]_{S0}$ (concentration value of (*S*)-form) and $[\mathbf{5}]_{R0}$ (concentration value of (*R*)-form). To this solution was added a MIP (46.0 mg, 13.0 μmol as recognizing sites), followed by stirring at 24 °C for 2 h. The MIP was filtered off, and the concentrate of this filtrate was defined as $[\mathbf{5}]_S$ and $[\mathbf{5}]_R$. $[\mathbf{5}]_0$ and [**5**] were determined by HPLC[15)] using *N*-*p*-toluenesulfonyl-2-aminoethanol (Ts-C2AA; **12**)[16)] as an internal standard. The adsorbed amount and selectivity were calculated using the following equations:

The adsorbed amount of the *S* form:

S_{ad} (%) = {($[\mathbf{5}]_0$ - $[\mathbf{5}]_S$) / $[\mathbf{5}]_0$} x 100

The adsorbed amount of the *R* form:

R_{ad} (%) = {($[\mathbf{5}]_0$ - $[\mathbf{5}]_R$) / $[\mathbf{5}]_0$} x 100

The enantiomeric excess in solution (% e.e.s.)

= {($[\mathbf{5}]_S$ - $[\mathbf{5}]_R$) / ($[\mathbf{5}]_S$ + $[\mathbf{5}]_R$)} x 100

The enantiomeric excess in the polymer (% e.e.p.)

$= \{(S_{ad} - R_{ad})/(S_{ad} + R_{ad})\} \times 100$

S_{ad} and R_{ad} were used as the indices of adsorbability, and e.e.p. was used as an index of selectivity.

The number of recognition sites in the MIP was calculated by dividing the number of template molecules (mmol) by the assumption of 100% of the yield of MIP (g), i.e.,

3 (mmol) / 10.54 (g) ≈ 0.285 (mmol/g)

In this paper, we calculated the equivalents of the MIPs based on this value.

Application: asymmetric amination

Preparation of *cat. A* and *cat. B*: (*RS*)-**9** (65.7 mg, 0.200 mmol) was dissolved in a mixture of cyclohexane, CH_2Cl_2 and EtOAc (153 mL; *c*-hex/ CH_2Cl_2/ EtOAc = 7/ 2/ 1). To this solution was added **IP-2** (3.50 g), which was stirred at r.t. for 30 min. The **IP-2** was filtered off (Kiriyama funnel (filter paper: No. 5B)) and washed with cyclohexane (15 mL). The combined filtrates were concentrated *in vacuo* to obtain unadsorbed **9**, with a slight incline to the (*R*)-isomer (7% *e.e.*) as a colorless solid. To the solid was added a mixed solvent of *n*-hexane and CH_2Cl_2 (4 mL; hexane/ CH_2Cl_2 (6: 4)), followed by filtration (minisalt SRP 15) to remove the racemate ((*RS*)-**9**) as a solid (95% recovered yield). The filtrate was concentrated *in vacuo* to afford (*R*)-**9** (1.5 mg) with 94% *e.e.* as a colorless solid that was used as ***cat. A***.

The filtered MIP was washed with MeOH (15 mL x 3) and CH_2Cl_2 (15 mL x 1). The eluent was concentrated *in vacuo*, followed by the same procedure as the filtered solution described above to give (*S*)-**9** (1.5 mg) with 94% *e.e.* as a colorless solid that was used as ***cat. B***.

The recovered MIP was reused after desiccation *in vacuo*, without further operation.

Asymmetric amination[14,17)]

The ***cat. A*** and $La(NO_3)_3 \cdot 6H_2O$ (4.40 mg, 10.0 mmol) were dissolved in EtOAc under an argon atmosphere. To the solution was added H-D-Val-O^{t}Bu in EtOAc (60.0 mL, 30.0 mmol) and ethyl 3-amino-3-oxo-2-phenylpropanoate **13** at r.t., and the reaction mixture was cooled to 0 °C. To the mixture was added di-*tert*-butyl azodicarboxylate **14** (55.3 mg, 0.240 mmol) in EtOAc (0.20 mL) at 0 °C with stirring for 48 hr. The reaction was quenched by the addition of 1M HCl aq. (1.0 mL), and the resultant mixture was extracted with CH_2Cl_2 (5.0 mL x 3). The organic layer was washed with satd. $NaHCO_3$ aq. (5.0 mL x 3), satd. NaCl aq. (5.0 mL x 3), and dried over anhyd. Na_2SO_4. After concentration *in vacuo*, the residue was purified by silica gel column chromatography (hexane/ EtOAc = 5: 1 to 3 :1) to give (*R*)-**15** (80.1 mg, 0.183 mmol, 92 %, 97 % *e.e.*) as a colorless foam: ^{1}H-NMR (300 MHz/$CDCl_3$) δ: 8.65 - 8.53 (1H, br m), 7.68 - 7.57 (2H, m), 7.47 - 7.28 (3H, m), 6.66 (1H, br s), 6.29 - 6.17 (1H, br m), 4.34 - 4.29 (1H, br m), 1.49 - 1.09 (21 H, m).

The same experiment using ***cat. B*** instead of ***cat. A*** gave (*S*)-**15** (86.6 mg, 0.198 mmol, 99 %, 97 % *e.e.*) as a colorless foam.

The enantiomeric excess of the obtained products was determined via HPLC; DAICEL CHIRALPAK AD-H, hexane/ MeOH= 95/ 5, 1.0 mL/min, 40 °C, 254 nm, t_R= 7.8 min (*S*), 10.4 min (*R*). Spectroscopic data was consistent with that previously reported.[17)]

Result and discussion

Evaluation of IP-2 and the solvent effect

We first evaluated the adsorbability of the racemic *N*-benzoyl-valine anilide ((*RS*)-**5**) onto **IP-2** in dichloromethane via batch procedure (Table 2). As described above, **IP-2** was prepared using hydrogen-bonding interactions as a main force in the pre-polymerization step and **IP-1** was similarly prepared using an acid-base interaction.[13)] Despite the lack of a stronger acid-base interaction that was advantageous for molding the recognition sites, surprisingly, **IP-2** showed a higher stereoselectivity than expected (entry 1). In CH_2Cl_2, *N*-benzoylvaline anilide exhibited lower adsorbability onto **IP-2** than that of a valine anilide onto **IP-1** (see Table 3, entry 2). Such a weak point for **IP-2**, however, was overcome by

decreasing the polarity of the solvent, and adequate adsorbability of **IP-2** was achieved. Comparing the results of adsorbability and enantioselectivity, we chose a mixed-solvent system that consisted of dichloromethane and *n*-hexane (1: 1) for further research (entry 2).

Table 2. The effects of solvents for the adsorbability of (*RS*)-**5** onto **IP-2**.

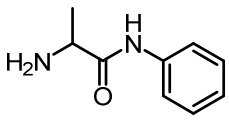
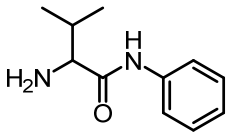
Entry	Solvent	S_{ad} (%)	R_{ad} (%)	e.e.p. (%)	e.e.s. (%)
1	CH_2Cl_2	25	12	35	8
2	CH_2Cl_2 : *c*-Hex = 50 : 50	46	30	21	13
3	CH_2Cl_2 : *c*-Hex = 25 : 75	64	54	8	12

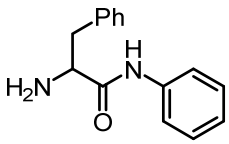
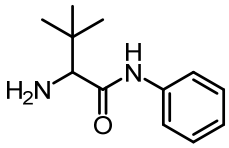
a) Conditions: (*RS*)-**5**, 0.26 mM, **IP-2** (5 eq), 24 °C, 2 hr.

Comparison of substrates

In our previous report, we found that **IP-1** synthesized using (*S*)-valine anilide as a template molecule, with the aid of a stronger acid-base interaction than hydrogen bonding, could recognize not only the enantiomers of a template molecule but also those of similar amino acid derivatives (Ala, Phe and *t*-Leu anilides), which showed similar enantioselectivities for all substrates, but also variations in the amount of adsorbed substrates (Table 3).[13)]

Table 3. Enantioselective adsorption of amino acid anilides **1**-**4** by **IP-1**.[a,b)]

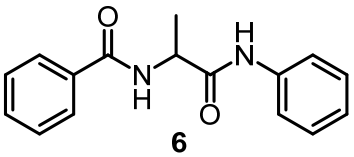
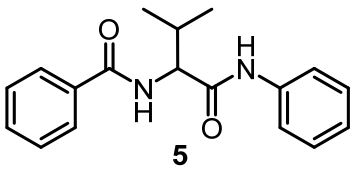
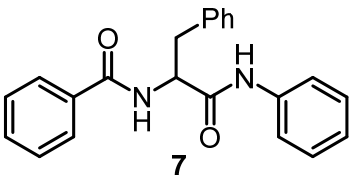
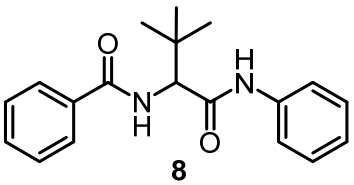
Entry	Substrate	S_{ad} (%)	R_{ad} (%)	e.e.p. (%)	e.e.s. (%)
1	 **2**	36	25	18	8
2	 **1**	45	30	20	12

3	 **3**	22	15	20	4
4	 **4**	38	26	18	9

a) Conditions: 0.26 mM, CH_2Cl_2, **IP-1** (3 eq), 24 °C, 2 hr.
b) See ref. 13.

Based on such curious results, we next evaluated the adsorbabilities and enantioselectivities of **IP-2** toward various types of *N*-benzoyl amino acid anilides (Table 4).

Table 4. Enantioselective adsorption of *N*-benzoyl amino acid anilides **5-8** by **IP-2**.[a)]

Entry	Substrate	S_{ad} (%)	R_{ad} (%)	e.e.p. (%)	e.e.s. (%)
1	 **6**	40	28	18	9
2	 **5**	46	30	21	13
3	 **7**	36	27	14	7
4	 **8**	38	26	19	9

a) Conditions: 0.26 mM, CH_2Cl_2/*c*-Hex = 1/1, **IP-2** (5 eq), 24 °C, 2hr.

To our delight, as shown in Table 4, ***IP-2*** *could effectively differentiate not only the enantiomers of template molecules, N-benzoyl valine anilide* ***5****, but also those of similar N-benzoyl amino acid derivatives* ***6, 7,*** *and* ***8*** *to yield slightly (R)-enriched solutions, along with preferentially (S)-adsorbed* ***IP-2****.* *N*-benzoyl phenylalanine anilide **7** possessed benzyl moieties that were larger than those of methyl, isopropyl, or *tert*-butyl groups in the amino acid scaffold and, intriguingly, it was adsorbed by **IP-2** in a ratio similar to other *N*-benzoyl amino acid anilides, but with an inferior level of enantioselectivity. The trend in these results concerning the adsorption of phenylalanine anilide onto **IP-2** was apparently different than the results with **IP-1**, which showed lower adsorbability and a similar enantioselectivity in the presence of acid-base interactions (see Table 3).

According to observations from the present study, the different properties of **IP-1** and **IP-2** with regard to adsorbability and enantioselectivity can be summarized as follows. The adsorbability of **IP-1** was strongly influenced by the basicity of amino acids. Phenylalanine has a lower basicity than other amino acids such as alanine, valine or *tert*-leucine[18], which is suspected to be caused by sterical bulkiness, and, therefore, the weaker acid-base interaction between the acidic moiety in the mold of a MIP and phenylalanine showed adsorption that was inferior to those of other amino acid anilides. However, once the amino acid anilides were attracted to the recognition sites of a MIP, the stronger acid-base interaction dominated the steric repulsion between the mold and the substrates, which then resulted in similar enantioselectivities for all the amino acid anilides we tested.

On the other hand, weak hydrogen-bonding attracting interactions and steric repulsion existed between the mold of **IP-2** and the *N*-benzoyl amino acid anilide substrates. Therefore, the steric repulsion between **IP-2** and the side chains of amino acids, particularly the benzyl group of phenylalanine, would affect the enantioselectivities for the adsorbance of **IP-2** in a more apparent fashion than that for **IP-1**, which introduces a slight decrease in the adsorbance of (*S*)-enantiomers to show a lower e.e.p. value. However, the strength of the hydrogen bonding between **IP-2** and *N*-benzoyl amino acid anilides seemed comparable, which would lead to similar adsorbabilities for amino acid derivatives toward **IP-2**.

Application

From our delightful results showing that MIPs molded using hydrogen bonding as a main attracting force caused an incline in the enantiomeric ratio, we envisaged the application of such a MIP to chiral amplification.[19-21] Thus, treatment of a solution of *racemic catalyst* to a MIP would cause a slight lean in the ratio of enantiomers, and the use of such an "enantioenriched solution" for asymmetric synthesis via chiral amplification could produce *enantiomerically pure compounds*. It is noteworthy that a racemic catalyst would produce optically pure compounds with the help of a MIP that would be easily recovered and recyclable.

Kumagai and Shibasaki, *et al*., previously reported the non-linear effect of chiral catalyst **9**[17] for an asymmetric Mannich-type reaction. In their report, the catalyst was prepared by intentional mixing of the pure enantiomers of (*S*)-**9** and (*R*)-**9**. Considering the structural similarities of **9** and **5**, we attempted to apply the above-mentioned methodology to the racemic solution of **9** for the generation of an enantioenriched solution of **9**, followed by application to an asymmetric Mannich-type reaction.

We first tried to prepare several types of MIPs for the enantioselective recognition of **9**, using various *N*-benzoyl-(*S*)-valine anilide derivatives (**10**, **11**) as chiral templates. Except for the use of **9**,[22] two types of MIPs, **IP-3** and **IP-4**, using **10** and **11** as template molecules, respectively, were newly synthesized (see Table 1).

Table 5. The effects of concentration and composition of the solvents for the enantioselective adsorption of (*RS*)-**9** by **IP-2**–**IP-4**.[a]

Entry	Conc. (mM)	MIP (eq)	Solvents (%)			S_{ad} (%)	R_{ad} (%)	e.e.p. (%)	e.e.s. (%)
			c-Hex	CH_2Cl_2	EtOAc[b]				
1	0.26	**IP-2** (5)	75	20	5	40	31	13	7
2	0.26	**IP-3** (5)	75	20	5	20	20	-	-
3	0.26	**IP-4** (5)	75	20	5	24	22	5	1
4	1.3	**IP-2** (1)	75	20	5	23	20	7	2
5	1.3	**IP-2** (3)	75	20	5	53	48	4	4

6	1.3	**IP-2** (5)	75	20	5	69	64	4	8
7	1.3	**IP-2** (5)	70	20	10	47	41	7	6
8	1.3	**IP-2** (5)	75	15	10	52	47	5	5

a) Conditions: Substrate was used (*RS*)-**9**, 24 ^{o}C, 2 hr.
b) See ref. 23.

The adsorbability and enantioselectivity of **IP-3** and **IP-4**, along with **IP-2**, was evaluated in the presence of (*RS*)-**9** as a substrate (Table 5, entries 1-3). As a result, contrary to our expectations, only **IP-2** gave satisfactory levels of adsorbability and enantioselectivity to a racemic solution of **9**, although its effect as a substrate was inferior to that of (*RS*)-**5**.

We next optimized the adsorption conditions (Table 5, entries 4-8). A greater concentration of the substrate (*RS*)-**5** led to a greater level of adsorption (entries 1 and 6). We also concluded that the addition of a 3-5 equivalent amount of IP-2 was optimal for the production of an adequate e.e.s. value (entries 4-6). The optimization of the composition of a solvent system was also determined, and an increase in the proportion of EtOAc decreased the amount of adsorption (entries 6-8).

Based on the results assembled in Table 5, the condition shown in entry 7 seemed to be the optimized choice for an appropriate adsorbability that would be compatible with enantioselectivity, and we applied this condition to the actual asymmetric Mannich-type amination system. Thus, the treatment of a racemic solution of **9** with 5 equivalents of **IP-2** at room temperature for 2 hours followed by separation gave the filtrate including a slightly (*R*)-inclined **9**, which was transformed into (*R*)-enriched **9** with 94% *e.e.* by single reprecipitation of the racemate. Moreover, adsorbed **9**, which was slightly inclined to an (*S*)-form, was also collected via the washing of **IP-2** with methanol, followed by the same procedure as the filtered solution described above to give (*S*)-enriched **9** with a 94% *e.e.* Both of the gained enantiomers, (*S*)-**9** and (*R*)-**9**, were applied to an asymmetric Mannich-type amination reaction, and, to our delight, both enantiomers gave the aminated products, (*R*)-**15** and (*S*)-**15**, respectively, in high yields with high enantioselectivities (Chart 1).

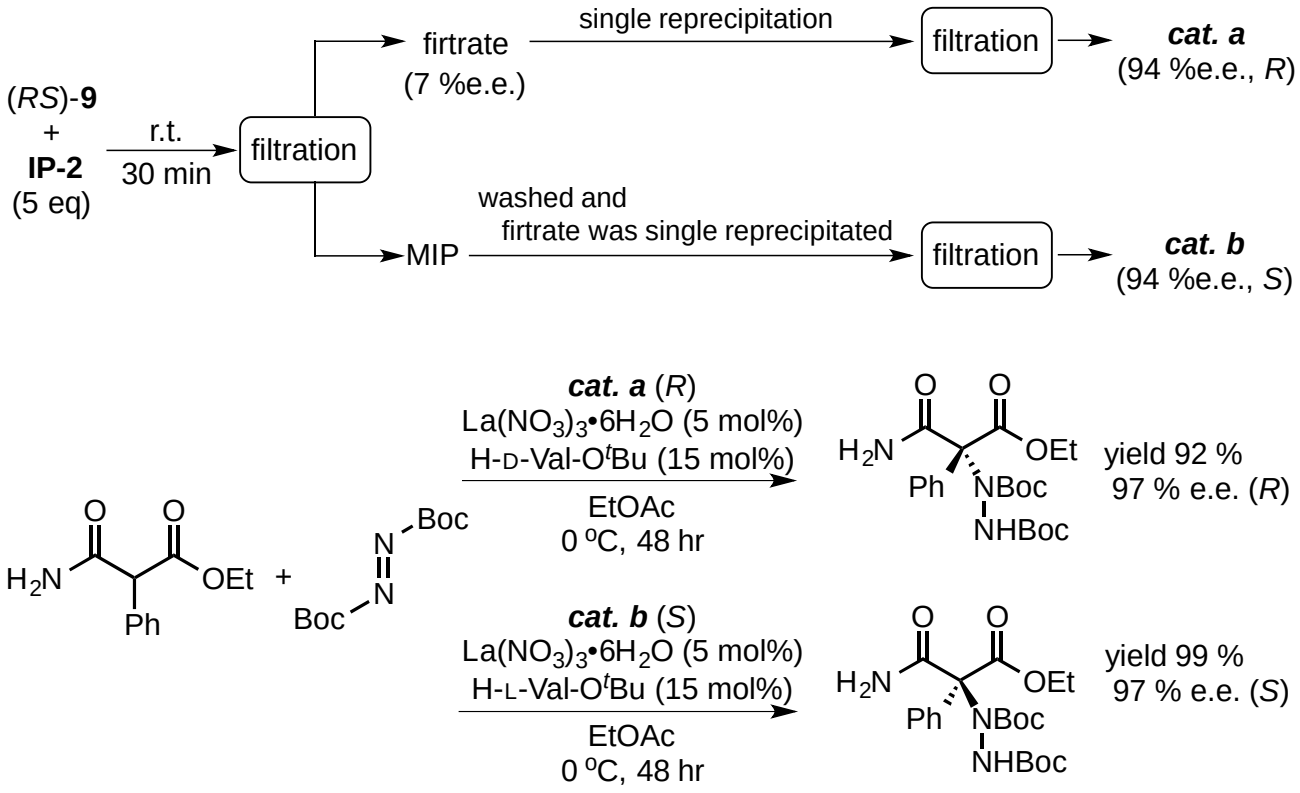


Chart 1. Application to chiral amplification.

Conclusions

We prepared a novel type of *N*-benzoyl-(*S*)-valine anilide-imprinted polymer, **IP-2**, using hydrogen-bonding interactions as a main force for molding and evaluated the details of the performance of **IP-2** via batch procedure. It appeared that **IP-2** could differentiate not only the enantiomer of a template molecule but also those of other types of *N*-benzoyl amino acid anilides, which led to the preferential adsorption of *N*-benzoyl-(*S*)-amino acid anilides in good order. Interestingly, the properties of **IP-2** differed from those of **IP-1**, which was synthesized using an acid-base interaction for the molding of adsorbability and enantioselectivity.

Based on such curious findings, we also found that **IP-2** could recognize the enantiomers of valine-derived bis(2-hydroxyphenyl)diamide **9**, the analogue of *N*-benzoylvaline anilide **5**, and that both (*R*)- and (*S*)-enriched **9** with 94% e.e. were easily afforded from the filtrate and filtered MIP, respectively, with the help of a single reprecipitation of the racemate. The (*R*)- and (*S*)-enriched **9** was applied to an asymmetric Mannich-type amination reaction, and the

corresponding aminated products were obtained in high yields with high enantioselectivities.

These intriguing results clearly prove that the treatment of a racemic catalyst with MIP triggered a highly stereoselective asymmetric synthesis via chiral amplification. Furthermore, adsorbed MIP was reusable after an appropriate work-up (washing with polar solvent, substitution to a non-polar solvent and desiccation).[24] Thus, we proved the great potential of a MIP for application to asymmetric syntheses and to recyclability for environmental friendliness.

Further, and more precise, mechanistic studies and synthetic applications to various asymmetric reactions are currently in progress.

Conflict of Interest

The authors declare no conflict of interest.

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15) HPLC condition were as follows; Table 2, table 4, entry 2: DAICEL CHIRALPAK IA, hexane-EtOH= 85: 15, 0.75 mL/min, 254 nm, **12** as internal standard, t_R= 8.8 min (*R*), 18.4 min (*S*), 27.8 min (internal standard); Table 4, entry 1: DAICEL CHIRALPAK IA, hexane-EtOH= 85: 15, 1.0 mL/min, 254 nm, **12** as internal standard, t_R= 9.2 min (*R*), 13.0 min (*S*), 19.6 min (internal standard); Entry 3: DAICEL CHIRALPAK IA, hexane-EtOH= 85: 15, 1.0 mL/min, 254 nm, **12** as internal standard, t_R= 13.7 min (*S*), 16.1 min (*R*), 19.4 min (internal standard); Entry 4: DAICEL CHIRALPAK IA, hexane-EtOH= 85: 15, 0.75 mL/min, 254 nm, **12** as internal standard, t_R= 6.7 min (*R*), 9.6 min (*S*), 25.7 min (internal standard);

Table 5: DAICEL CHIRALPAK IC, hexane-EtOH= 95/5, 0.75 mL/min, 254 nm, *N*-benzoyl-2-phenethylamine as internal standard, t_R= 11.8 min (*S*), 13.4 min (*R*), 26.1 min (internal standard).

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18) Predicted pKa values of the protonated amines shown in SciFinder (calculated by ACD/Labs); phenylalanine: 9.20±0.15; alanine: 9.61±0.13; valine: 10.21±0.25; *tert*-leucine: 10.51±0.38.

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22) Unfortunately, compound (*RS*)-**9** did not tolerated radical polymerization condition and we could not prepare the corresponding MIP.

23) (*RS*)-**9** could not dissolve in non-polar solvent without polar co-solvent. For this reason, ethyl acetate was added in the presence of (*RS*)-**9** as a substrate.

24) The recyclability of IP-2 is shown in Table 6. These results were part of the adsorption experiments of (*RS*)-**9** using IP-2, as shown in Chart 1.

Table 6. Recyclability of IP-2[a)]

Run	S_{ad} (%)	R_{ad}(%)	e.e.s. (%)	e.e.p. (%)
1	46	39	8	7
2	44	36	9	7
3	44	37	9	6

a) Conditions: (*RS*)-**9** (0.20 mmol), IP-2 (5 eq), *c*-Hex/ CH_2Cl_2/ EtOAc = 7/ 2/ 1, r.t., 30 min.

Through the recycling process, none of the turbidity by the pulverization of the polymer particle in the filtrate was found. Considering this observation and the results of Table 6, we concluded that IP-2 was reusable.