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Efficient resolution of racemic 1,1′-bi-2-naphthol with chiral selectors identified from a small library

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Abstract

Efficient resolution of racemic 1,1′-bi-2-naphthol, a well-studied analyte in chiral separation, was achieved using selectors developed from a small library. Separation factors (up to 7.2) obtained are significantly higher than the ones reported previously for this analyte. The library consists of 121 members and it does not contain the pi deficient 3,5-dinitrobenzoyl (Dnb) group. These highly efficient stationary phases may lead to the practical large-scale chromatographic resolution of enantiomers of 1,1′-bi-2-naphthol, which are widely used as chiral auxiliaries and ligands in asymmetric synthesis.

Keywords: Combinatorial library; Enantiomer separation; 1,1′-Bi-2-naphthol

1. Introduction

As enzymes and other biological receptor molecules possess chiral structures, enantiomers of a racemic compound may be absorbed, activated, and degraded by them in different manners. Due to this phenomenon, in many instances, two enantiomers of a racemic drug may have different or even opposite pharmacological activities. In order to acknowledge these differing effects, the biological activity of each enantiomer often needs to be studied separately. This and other factors within the pharmaceutical industry have contributed significantly to the need for enantiomerically pure compounds and thus the need for chiral chromatography [1].

In order to develop efficient enantioselective stationary phases, several groups including ours have investigated the development of chiral selectors using chemical library approaches [2–11]. Key requirements to the success of the library methods are the generation of a large number of compounds to be studied and an efficient method to evaluate the properties of these compounds. Libraries can be generated combinatorially (combinatorial libraries), or they can be prepared non-combinatorially. The library can be a mixture of compounds (mixture library) or it can be a collection of pure compounds in individual containers (parallel library) [12]. Feasibility of the library method in chiral selector development has been demonstrated by several groups. However, its practical application in chiral separation is still yet to be proved. In many of the reported examples including our own, significant chiral separation of the chosen analytes or their close analogues had already been achieved.

In this paper, we would like to report the efficient resolution of racemic 1,1′-bi-2-naphthol (Fig. 1) using the library approach. 1,1′-Bi-2-naphthol is one of the most popular analytes in chiral separation and has been studied extensively [13–19]; therefore, our results could be evaluated with reference to literature precedence. We avoided 3,5-dinitrobenzoyl (Dnb) group in our library design in order to demonstrate more convincingly the practical utility of this library screening approach. Since the analyte 1,1′-bi-2-naphthol does not contain a 3,5-dinitrobenzoyl group, this is the first combinatorial example from our laboratory that does not rely on the role of a 3,5-dinitrobenzoyl group in chiral separation.

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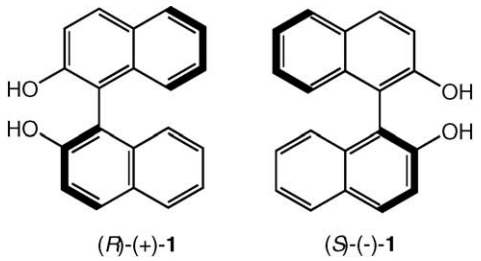


Fig. 1. *R* and *S* enantiomers of racemic 1,1′-bi-2-naphthol.

It should also be pointed out that efficient chiral resolution of 1,1′-bi-2-naphthol is also of significant practical importance. Optically active 1,1′-bi-2-naphthol (**1**) and its derivatives are frequently used as chiral auxiliaries and ligands in asymmetric synthesis [20]. Even though asymmetric synthesis of this compound has been reported, this important material is generally obtained in enantiomerically pure forms by resolution of the racemic mixture. Of the resolution methods that have been reported, the popular procedure involves three steps: (1) the conversion of the racemic 1,1′-bi-2-naphthol to its phosphates, (2) resolution of the phosphates via its cinchonine salts, and (3) re-conversion of the resolved phosphate salts back to 1,1′-bi-2-naphthol [20]. Highly efficient enantioselective stationary phases could provide a viable alternative to the rather tedious derivative fractional crystallization procedure.

2. Experimental

2.1. Abbreviations

DIC, diisopropylcarbodiimide; HOBt, 1-hydroxybenzotriazole; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; DIPEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DCM, dichloromethane; Boc, *tert*-butyloxycarbonyl; Fmoc, 9-fluorenylmethoxycarbonyl; Hyp: *trans*-4-hydroxyproline; Tic: tetrahydroisoquinoline-3-carboxylic acid; (Me)Val: *N*-methyl valine; Phg: phenylglycine; (Me)Ahx: 6-methylaminohexanoic acid; AmPS: aminomethylated polystyrene resin.

FmocOSu, 9-fluorenylmethyloxycarbonyl-*N*-hydroxysuccinimide; Fmoc-(Me)Ahx-OH, 6-[(9H-fluoren-9-ylmethoxy)carbonyl]methylaminohexanoic acid; Fmoc-Ahx-OH, 6-[(9H-fluoren-9-ylmethoxy)carbonyl]aminohexanoic acid; Fmoc-His(Trt)-OH, *N*-α-Fmoc-*N*-im-trityl-L-histidine; Fmoc-Asn(Trt)-OH, *N*-α-Fmoc-*N*-β-trityl-L-asparagine; Fmoc-D-Asn(Trt)-OH, *N*-α-Fmoc-*N*-β-trityl-D-asparagine; Fmoc-Hyp(tBu)-OH, *N*-α-Fmoc-*O*-*t*-butyl-L-trans-4-hydroxyproline; Fmoc-Trp(Boc)-OH, *N*-α-Fmoc-*N*-in-*t*-Boc-L-tryptophan; Fmoc-Thr(tBu)-OH, *N*-α-Fmoc-*O*-*t*-butyl-L-threonine; Fmoc-D-Thr(tBu)-OH, *N*-α-Fmoc-*O*-*t*-butyl-D-threonine [2R, 3S]; Fmoc-Asp(OtBu)-OH, *N*-α-Fmoc-L-aspartic acid β-*t*-butyl ester.

2.2. General supplies and equipment

Amino acid derivatives were purchased from NovaBiochem (San Diego, CA, USA). All other chemicals and solvents were purchased from Aldrich (Milwaukee, WI, USA), Fluka (Ronkonkoma, NY, USA), or Fisher Scientific (Pittsburgh, PA, USA). HPLC grade Kromasil® silica gel (particle size 5 μm, pore size 100 Å, and surface area 298 m^2/g) was purchased from Akzo Nobel (EKA Chemicals, Bohus, Sweden). Selecto silica gel (32–63 μm) from Fisher Scientific was used for flash column chromatographic purification of target compounds. Thin-layer chromatography was completed using EM silica gel 60 F-254 TLC plates (0.25 mm; E.Merck, Darmstadt, Germany). The HI-TOP manual synthesizer required for parallel library synthesis is from Whatman Polyfiltronic (Rockland, MA, USA). Elemental analyses were conducted by Atlantic Microlab Inc. (Norcross, GA, USA). HPLC analyses were completed with a Beckman analytical gradient system (System Gold). UV spectra were obtained with a Shimadzu UV 201 spectrometer (cell volume 3 mL; cell pass length 10 mm).

2.3. Preparation of Fmoc-(Me)Ahx-OH

A solution of *N*-methylcaprolactam (12.72 g, 100 mmol) in 200 mL of concentrated hydrochloric acid was stirred at RT for 1 h, then heated to reflux for 24 h. The solution was extracted with ethyl acetate. Crude product obtained after evaporation of the aqueous layer under vacuum was re-crystallized using a mixture of ethanol and ether. 6-Methylaminohexanoic acid hydrochloride was isolated as a white solid in 88% yield, m.p. 66–67 °C, ^{1}H NMR (D_2O): δ 1.31 (m, 2H), 1.57 (m, 4H), 2.31 (t, J = 7.3 Hz, 2H), 2.60 (s, 3H), 2.93 (t, J = 7.6 Hz, 2H).

To a stirred solution of 6-methylaminohexanoic acid hydrochloride (6.36 g, 35 mmol) and sodium hydroxide (2.80 g, 70 mmol) in 100 mL of water was added gradually Fmoc-OSu (10.1 g, 30 mmol) in 100 mL of THF. After being stirred at room temperature for 8 h, the reaction mixture was acidified to pH 2 with concentrated hydrochloric acid. The solution was extracted with ethyl acetate (3× 100 mL), and the organic extracts were washed with water and then dried over anhydrous sodium sulfate. Evaporation of the solvent yielded the crude product, which was purified by flash column chromatography on silica gel (mobile phase 10% methanol in DCM) to give the desired compound as a colorless oil (9.04 g, 82% yield). ^{1}H NMR (CD_2Cl_2): δ 1.1–1.7 (m, 4H), 2.37 (m, 2H), 2.8–3.4 (m, 5H), 4.2–4.6 (m, 3H), 7.2–7.9 (m, 8H). ESI–MS: m/z 368.3 ($M + H^+$).

2.4. Preparation of (Me)Ahx-AmPS

To a 50 mL peptide synthesis vessel were added AmPS resin (5.1 g, 1.84 mmol of amine group), Fmoc-(Me)Ahx-OH (5 equiv., 3.38 g), HOBt (3 equiv., 0.85 g) in 15 mL of DMF and 15 mL of DCM. After shaking for 5 min, DIC (5 equiv.,

1.16 g) was added and then the mixture was agitated at RT for 10 h. The resin was collected by filtration and washed with DMF, methanol, DCM and methanol (20 mL × 3). The surface (Me)Ahx concentration was determined to be 0.38 mmol/g based on the Fmoc cleavage method [21]. The Fmoc protecting group was then removed by treatment of the resin with 30 mL of 20% (v/v) piperidine in DMF for 1 h. The deprotected resin, (Me)Ahx-AmPS, was collected by filtration and washed with DMF, methanol, DCM and methanol (20 mL × 3).

2.5. Preparation of the parallel 121-member library

The library was synthesized using the polyfiltronic HI-TOP manual synthesizer. 30 mg of the (Me)Ahx-AmPS resin synthesized above was transferred to each of the 121 wells of two 96-well filter microplates. Each library member was then synthesized individually. The experimental procedure for the synthesis of the Fmoc-Asn(Trt)-Asn(Trt) member of the library is shown below. Other library members were prepared following a similar sequence. To 30 mg (0.011 mmol in (Me)Ahx group) of (Me)Ahx-AmPS resin prepared above in one well of the 96-well filter microplate were added mixtures of Fmoc-Asn(Trt)-OH (14.3 mg, 2 equiv.), HATU (9.1 mg, 2 equiv.), and DIPEA (3.1 mg, 2 equiv.) in 0.8 mL of DMF. After agitating for 6 h, the resulting resin was filtered and washed with DMF, methanol, DCM and methanol. The above coupling cycle was repeated one more time with only one equivalent of Fmoc-Asn(Trt)-OH, HATU, and DIPEA in 0.8 mL of DMF. The Fmoc protecting group was then removed by treatment with 0.8 mL 20% (v/v) piperidine in DMF for 1 h, followed by washing with DMF, methanol, DCM and methanol. Then the second module member, Fmoc-Asn(Trt)-OH, was coupled to the resulting resin following an identical reaction sequence to yield the desired library member on the AmPS resin.

2.6. Screening of the parallel library with chiral HPLC

A stock solution (0.105 mg/mL of 1,1′-bi-2-naphthol) of racemic 1,1′-bi-2-naphthol was made by adding 42.1 mg racemic 1,1′-bi-2-naphthol to 200 mL 1,2-dichloroethane and 200 mL *n*-heptane. To each well of the parallel 121-member library was added 400 μL of 1,2-dichloroethane to swell the resin for 30 min, then was added 600 μL of the stock solution. After incubating the mixture for 3 h, the supernatants were filtered into a collection plate using the Polyfiltronic filtration manifold. 400 μL of the resulting supernatant solution in each well was transferred into the sample vials used for HPLC autosampler. The enantiomeric ratios of the supernatant were analyzed by HPLC using a homemade Dnb-Pro-Pro-Aun-APS column (column size, 50 mm × 4.6 mm; mobile phase, 60% IPA in *n*-hexane; flow rate, 1.0 mL/min; UV detection at 254 nm), which yielded a separation factor of 1.43 (t_0 = 0.567, t_1 = 4.400, t_2 = 6.033 min).

2.7. Preparation of the chiral stationary phase of Fmoc-Asn(Trt)-Asn(Trt)-(Me)Ahx-APS

APS (3-aminopropyl silica gel) was prepared from Kromasil® silica gel (5 μm spherical silica, 100 Å, 298 m^2/g) and 3-aminopropyltriethoxysilane. The surface amino concentration is 0.66 mmol/g, based on elemental analysis data of nitrogen (C, 3.11; H, 0.83; N, 0.93).

To a 50 mL peptide synthesis vessel were added APS (8.0 g, 5.3 mmol of amine group), Fmoc-(Me)Ahx-OH (3 equiv., 5.85 g), HOBt (1 equiv., 0.81 g) in 10 mL of DMF and 10 mL of DCM. After shaking for 5 min, DIC (3 equiv., 2.01 g) was added and then the mixture was agitated at RT for 8 h. The silica was collected by filtration and washed with DMF, methanol, and DCM (20 mL × 3). The above coupling cycle was repeated one more time. The unreacted free amine group on the silica gel was end-capped by reacting with acetic anhydride and pyridine in DCM (3:2.5:30, v/v/v). The surface (Me)Ahx concentration was determined to be 0.64 mmol/g based on the Fmoc cleavage method. The Fmoc protecting group was then removed by treatment of the silica with 20 mL of 20% (v/v) piperidine in DMF for 1 h. The deprotected silica, (Me)Ahx-APS, was collected by filtration and washed with DMF, methanol, and DCM (20 mL × 3).

To 0.80 g of (Me)Ahx-APS silica (the surface (Me)Ahx concentration was 0.64 mmol/g) were added mixtures of Fmoc-Asn(Trt)-OH (3 equiv., 0.92 g), HATU (3 equiv., 0.58 g), and DIPEA (3 equiv., 0.20 g) in 8 mL of DMF. After agitating for 6 h, the resulting silica was filtered and washed with DMF, methanol, and DCM. The surface Asn(Trt) concentration was determined to be 0.44 mmol/g based on the Fmoc cleavage method. The Fmoc protecting group was then removed by treatment of the silica with 10 mL of 20% (v/v) piperidine in DMF for 1 h. The deprotected silica, Asn(Trt)-(Me)Ahx-APS, was collected by filtration and washed with DMF, methanol, and DCM. Then another module, Fmoc-Asn(Trt)-OH, was coupled to the resulting silica following a identical reaction sequence. The surface Fmoc concentration was determined to be 0.39 mmol/g based on the Fmoc cleavage method. After that, the remaining unreacted free amine groups in the coupling reactions were end-capped by reacting with acetic anhydride and pyridine in DCM, and washed with DMF, methanol, and DCM to yield the desired chiral selector on the silica gel. The resulting chiral stationary phase was packed into a 50 mm × 4.6 mm HPLC column using a standard slurry packing method.

2.8. Preparation of the chiral stationary phase of Fmoc-Asn(Trt)-Asn(Trt)-Ahx-APS

The stationary phase was prepared by using Fmoc-Ahx-OH instead of Fmoc-(Me)Ahx-OH as the linker. Otherwise, the procedure is identical to the one described for the preparation of the chiral stationary phase of Fmoc-Asn(Trt)-Asn(Trt)-(Me)Ahx-APS.

Fmoc concentration of the linker coupling step was 0.60 mmol/g; Fmoc concentration of the first Asn(Trt) coupling was determined to be 0.51 mmol/g; and Fmoc concentration of the second Asn(Trt) coupling was determined to be 0.37 mmol/g.

2.9. Preparation of the chiral stationary phase of Fmoc-D-Thr(tBu)-Hyp(tBu)-(Me)Ahx-APS

Fmoc concentration of the linker coupling step was 0.64 mmol/g; Fmoc concentration of the Hyp(tBu) coupling was determined to be 0.54 mmol/g; and Fmoc concentration of the D-Thr(tBu) coupling was determined to be 0.49 mmol/g.

2.10. Preparation of the chiral stationary phase of Fmoc-D-Thr(tBu)-Tic-(Me)Ahx-APS

Fmoc concentration of the linker coupling step was 0.64 mmol/g; Fmoc concentration of the Tic coupling was determined to be 0.55 mmol/g; and Fmoc concentration of the D-Thr(tBu) coupling was determined to be 0.44 mmol/g.

2.11. Chromatographic measurements

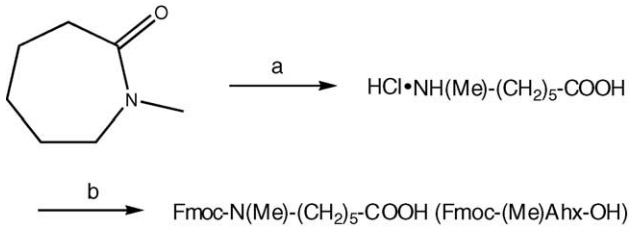
Retention factor (k) equals $(t_r - t_0)/t_0$ in which t_r is the retention time and t_0 is the dead time. Dead time t_0 was measured with 1,3,5-tri-*t*-butylbenzene as the void volume marker. Flow rate at 1 mL/min., UV detection at 254 nm.

3. Results and discussion

The library designed is a dipeptide library consisting of two amino acid modules (Fig. 2). The two modules are identical, both contain the same eleven amino acids. All the possible combinations of two modules yield a library containing a total of 121 members. The library differs from previous libraries used in this laboratory in several aspects. First of all, 3,5-dinitrobenzoyl group is avoided in this library design. Secondly, unlike previous libraries prepared in this laboratory, the side chain protecting groups of amino acids are kept as part of the chiral selectors, as their steric hindrance may amplify chiral recognition. Thirdly, a new linker, 6-methylaminohexanoic acid, is used in place of the 4-aminobutyric acid (Abu) linker used previously in this laboratory. This new linker does not introduce

Fmoc-[His(Trt), Asn(Trt), D-Asn(Trt), Hyp(tBu), Trp(Boc), Thr(tBu), D-Thr(tBu), Tic, (Me)Val, Phg, Asp(tBu)]$_2$-(Me)Ahx-AmPS

Fig. 2. The 121-member library. Fmoc, 9-fluorenylmethoxycarbonyl; Trt: trityl; tBu: tert-butyl; Boc: tert-butyloxycarbonyl; Hyp: *trans*-4-hydroxyproline; Tic: tetrahydroisoquinoline-3-carboxylic acid; (Me)Val: *N*-methyl valine; Phg: phenylglycine; (Me)Ahx: 6-methylaminohexanoic acid ($MeNH(CH_2)_5CO_2H$); AmPS: aminomethylated polystyrene resin. Unless indicated otherwise, amino acids adopt the L-configuaration.



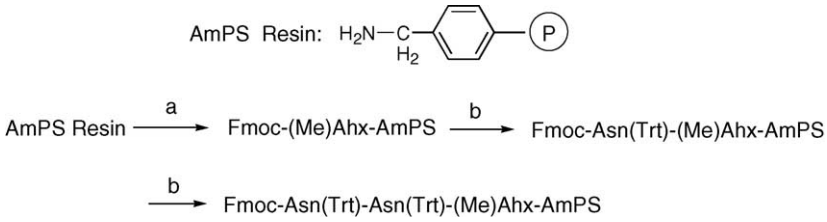
Scheme 1. Preparation of Fmoc protected 6-methylaminohexanoic acid (Fmoc-(Me)Ahx-OH). (a) HCl (conc.), reflux; (b) Fmoc-OSu.

a hydrogen-bonding donor NH group next to the chiral selector. It was introduced to minimize attractive interactions with the analyte that are common to all library members. The Fmoc protected form of this amino acid was prepared from *N*-methylcaprolactam according to Scheme 1. Structural diversity of the library is achieved by the application of amino acids that are significantly different from each other, including *N*-methylamino and D-amino acids [22].

The resin chosen for the synthesis of this library is aminomethylated polystyrene (AmPS) resin, a derivative of the widely used Merrifield resin. The synthesis of this library was performed using a Hi-top filter plate manual synthesizer as described in our previous publication [21]. In terms of the chemistry involved, Fmoc solid phase synthesis with the powerful coupling reagent HATU was chosen [23], and the detailed chemistry is illustrated in Scheme 2 with the synthesis of the Fmoc-Asn(Trt)-Asn(Trt) member of the library. In this synthesis, the commercially available, side chain protected form of Asn, *N*-α-Fmoc-*N*-β-trityl-asparagine (Fmoc-Asn(Trt)-OH) is needed. Other library members were synthesized following similar reactions.

For screening purpose, an equal amount of the racemic analyte (0.063 mg, 0.00022 mmol) in a mixture of 1,2-dichloroethane and *n*-heptane (7:3, 1.00 ml) was added to each of the wells that contained 0.011 mmol of the selector. After equilibration for 3 h, the supernatants were collected into a collection plate. Enantiomeric ratio of the supernatant was analyzed using a Dnb-Pro-Pro-Aun-APS column prepared previously in the laboratory, which yielded a separation factor of 1.4. Ratio of the two peak areas was used as the relative measure of the enantiomeric purity. The measured ratios of peak areas obtained for the supernatants for all 121 wells of the library are summarized in Table 1.

Since separation factor = K_B/K_A, one can estimate separation factors based on the data obtained in the screening experiment. It can be shown that separation factor = $K_B/K_A = (Cs/Cm)_B/(Cs/Cm)_A = (Cs)_B/(Cs)_A \times (Cm)_A/(Cm)_B = (Cm^0 - Cm)_B/(Cm^0 - Cm)_A \times (Cm)_A/(Cm)_B = (PA^0 - PA)_B/(PA^0 - PA)_A \times (PA)_A/(PA)_B = (PA^0/PA - 1)_B/(PA^0/PA - 1)_A$, where Cm^0 and PA^0 are the concentration of the enantiomer in the supernatant and the peak area of the enantiomer in the analysis of the supernatant before incubation. Cm and PA are the corresponding values after the incubation. These estimated separation factors are listed in Table 1 along with the peak area ratios. Some of estimated



Scheme 2. Preparation of the Fmoc-Asn(Trt)-Asn(Trt) member of the parallel library: (a) Fmoc-(Me)Ahx-OH, DIC. (b) (1) piperidine; (2) Fmoc-Thr(tBu)-OH, HATU.

Table 1
Ratios of peak areas obtained for the supernatants for the 121 members of the library and the estimated separation factors (in parenthesis)

His-His	His-Asn	His-D-Asn	His-Hyp	His-Trp	His-Thr	His-D-Thr	His-Tic	His-MeVal	His-Phg	His-Asp
1.33 (1.46)	1.36 (1.49)	1.36 (1.49)	1.35 (1.48)	1.34 (1.48)	1.30 (1.43)	1.30 (1.43)	1.34 (1.46)	1.06 (1.12)	1.00 (1.03)	0.99 (1.01)
Asn-His	Asn-Asn	Asn-D-Asn	Asn-Hyp	Asn-Trp	Asn-Thr	Asn-D-Thr	Asn-Tic	Asn-MeVal	Asn-Phg	Asn-Asp
1.45 (1.81)	2.04 (4.17)	0.97 (0.98)	1.54 (2.53)	0.86 (0.71)	0.98 (1.01)	0.97 (0.96)	1.15 (1.45)	1.00 (1.06)	0.96 (0.95)	1.00 (1.08)
D-Asn-His	D-Asn –Asn	D-Asn-D-Asn	D-Asn-Hyp	D-Asn-Trp	D-Asn-Thr	D-Asn-D-Thr	D-Asn-Tic	D-Asn-MeVal	D-Asn-Phg	D-Asn-Asp
0.98 (1.01)	1.02 (1.10)	0.51 (0.31)	0.97 (0.99)	0.94 (0.90)	0.98 (1.01)	0.97 (0.98)	0.95 (0.93)	0.95 (0.93)	0.97 (0.97)	0.96 (0.93)
Hyp-His	Hyp-Asn	Hyp-D-Asn	Hyp-Hyp	Hyp-Trp	Hyp-Thr	Hyp-D-Thr	Hyp-Tic	Hyp-MeVal	Hyp-Phg	Hyp-Asp
1.55 (1.82)	0.95 (0.95)	0.96 (1.00)	0.98 (1.00)	1.01 (1.07)	0.98 (1.00)	2.10 (3.68)	0.99 (1.03)	0.95 (0.93)	1.58 (2.33)	0.99 (1.03)
Trp-His	Trp-Asn	Trp-D-Asn	Trp-Hyp	Trp-Trp	Trp-Thr	Trp-D-Thr	Trp-Tic	Trp-MeVal	Trp-Phg	Trp-Asp
1.13 (1.27)	0.97 (0.97)	0.97 (0.99)	0.99 (1.02)	0.98 (1.00)	0.98 (1.02)	0.98 (1.00)	0.95 (0.91)	0.93 (0.83)	0.97 (0.97)	0.97 (0.96)
Thr-His	Thr-Asn	Thr-D-Asn	Thr-Hyp	Thr-Trp	Thr-Thr	Thr-D-Thr	Thr-Tic	Thr-MeVal	Thr-Phg	Thr-Asp
1.19 (1.36)	0.95 (0.91)	0.95 (0.89)	1.00 (1.07)	1.01 (1.11)	0.97 (0.94)	1.01 (1.19)	0.97 (0.97)	0.96 (0.93)	1.00 (1.10)	0.95 (0.89)
D-Thr-His	D-Thr-Asn	D-Thr-D-Asn	D-Thr-Hyp	D-Thr-Trp	D-Thr-Thr	D-Thr-D-Thr	D-Thr-Tic	D-Thr-MeVal	D-Thr-Phg	D-Thr-Asp
1.21 (1.42)	1.01 (1.15)	1.00 (1.07)	1.08 (1.35)	0.91 (0.83)	0.94 (0.83)	0.98 (1.05)	1.00 (1.10)	0.95 (0.89)	0.96 (0.92)	0.97 (0.97)
Tic-His	Tic-Asn	Tic-D-Asn	Tic-Hyp	Tic-Trp	Tic-Thr	Tic-D-Thr	Tic-Tic	Tic-MeVal	Tic-Phg	Tic-Asp
1.18 (1.32)	0.95 (0.92)	0.94 (0.85)	0.98 (1.00)	0.97 (0.95)	0.96 (0.91)	1.40 (3.57)	0.96 (0.94)	0.96 (0.94)	0.98 (0.99)	0.95 (0.92)
MeVal-His	MeVal-Asn	MeVal-D-Asn	MeVal-Hyp	MeVal-Trp	MeVal-Thr	MeVal-D-Thr	MeVal-Tic	MeVal-MeVal	MeVal-Phg	MeVal-Asp
0.97 (0.98)	1.03 (1.21)	0.92 (0.80)	1.00 (1.11)	0.98 (1.03)	0.99 (1.09)	0.98 (1.06)	0.99 (1.05)	0.98 (1.00)	0.99 (1.07)	0.99 (1.67)
Phg-His	Phg-Asn	Phg-D-Asn	Phg-Hyp	Phg-Trp	Phg-Thr	Phg-D-Thr	Phg-Tic	Phg-MeVal	Phg-Phg	Phg-Asp
1.11 (1.26)	1.00 (1.10)	0.94 (0.86)	1.03 (1.24)	0.97 (0.98)	0.97 (0.89)	0.99 (1.11)	0.99 (1.11)	0.97 (0.98)	0.98 (1.00)	0.97 (0.63)
Asp-His	Asp-Asn	Asp-D-Asn	Asp-Hyp	Asp-Trp	Asp-Thr	Asp-D-Thr	Asp-Tic	Asp-MeVal	Asp-Phg	Asp-Asp
1.10 (1.26)	1.02 (1.18)	0.95 (0.85)	0.99 (1.03)	0.96 (0.89)	0.97 (0.88)	0.98 (1.05)	0.97 (0.97)	0.95 (0.84)	0.98 (1.04)	0.94 (0.80)

Library template: AmPS-Ahx(Me)-AA1-AA2-Fmoc. Due to space limitation, protecting groups of amino acids are not shown. Ratio of the peak areas of racemic mixture **1** is 0.98.

values are lower than one, indicating reversed selectivity. In our experimental design, these estimated separation factors may contain considerable errors, as the solid supports used in the screening experiment and the subsequent chromatographic experiment are different. Nonetheless, the estimated separation factors should be a better indicator of enantioselectivity than just the peak area ratio, which not only depends on the enantioselectivity but also depends on the degree of adsorption.

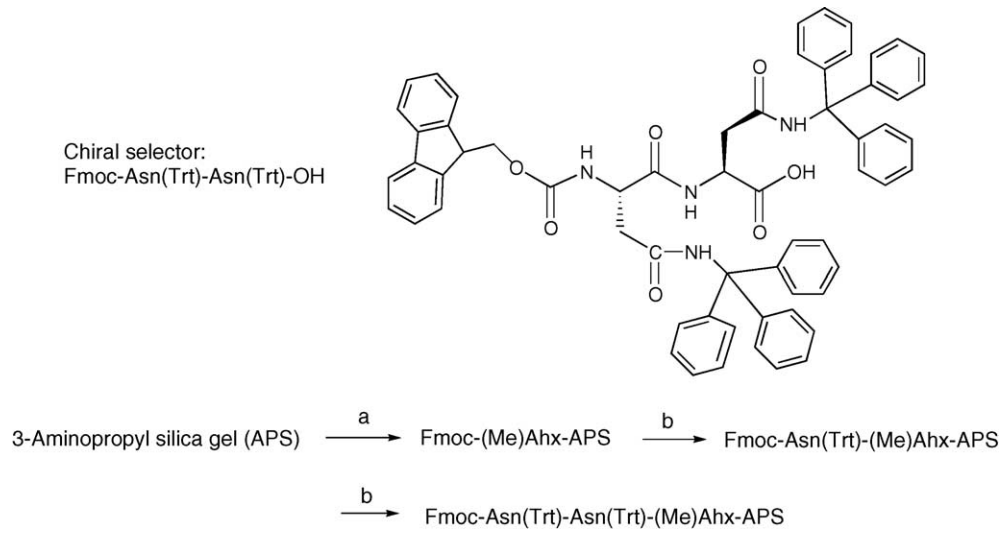
It is apparent from these estimated separation factors, several promising leads exist in the library. The most promising selector is Fmoc-Asn(Trt)-Asn(Trt) as judged by these estimated separation factors. It was thus chosen for the chromatographic resolution of racemic 1,1′-bi-2-naphthol. For this purpose, this chiral selector is immobilized onto silica gel with two different linkers, 6-aminohexanoic acid and 6-methylaminohexanoic acid. The relatively long linker serves to minimize impact of the polar surface of silica gel on chiral separation [24]. The stationary phases are prepared according to Scheme 3. The resulting stationary phases were then packed into columns using the standard slurry packing method. For comparison, the second and third best selectors [Fmoc-D-Thr(tBu)-Hyp(tBu); Fmoc-D-Thr(tBu)-Tic] based on these estimated separation factors were also immobilized onto silica gel using the 6-methylaminohexanoic acid linker.

Table 2
Chiral resolution of racemic 1,1′-Bi-2-naphthol **1**

Chiral selector	Linker	k_r	α	Surface loading (mmol/g)
Fmoc-Asn(Trt)-Asn(Trt)	Ahx	4.0	7.2	0.37
	(Me)Ahx	2.6	4.7	0.39
Fmoc-D-Thr(tBu)-Hyp(tBu)	(Me)Ahx	1.6	2.6	0.49
Fmoc-D-Thr(tBu)-Tic	(Me)Ahx	1.5	2.3	0.44

k_r: Retention factor of the least retained enantiomer. Mobile phase: CH_2Cl_2.

Separation factors obtained for these stationary phases are summarized in Table 2. Excellent resolution of the racemic 1,1′-bi-2-naphthol was achieved with all the columns prepared, with separation factors as high as 7.2 (Fig. 3). Selector Fmoc-Asn(Trt)-Asn(Trt) is more efficient than selectors Fmoc-D-Thr(tBu)-Hyp(tBu) and Fmoc-D-Thr(tBu)-Tic. The



Scheme 3. Preparation of the Fmoc-Asn(Trt)-Asn(Trt)-(Me)Ahx stationary phase. (a) Fmoc-(Me)Ahx-OH, DIC. (b) (1) piperidine; (2) Fmoc-Asn(tBu)-OH, HATU. Other stationary phases are prepared similarly.

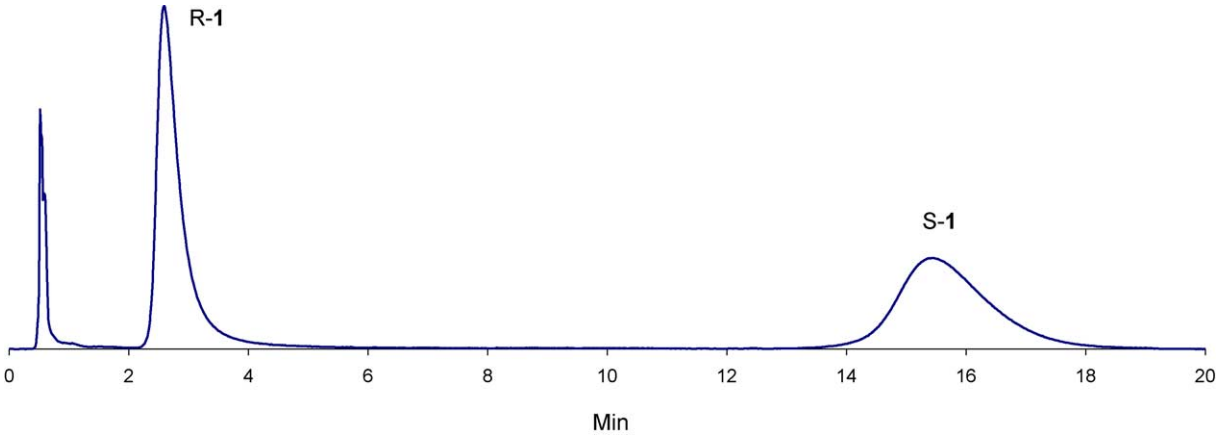


Fig. 3. Chiral resolution of 1,1′-bi-2-naphthol (**1**) with Fmoc-Asn(Trt)-Asn(Trt)-Ahx-NH$(CH_2)_3$ Silica. First eluting peak was due to the dead volume marker.

separation factors for chiral selector Fmoc-Asn(Trt)-Asn(Trt) depend on the choice of the linker [24], with the simpler 6-aminohexanoic acid linker being more effective.

The chiral resolution of racemic 1,1′-bi-2-naphthol has been studied extensively. Most separation factors reported for this analyte are less than 2.5. So far, we found only one separation factor (4.2) [14] that is close to but still lower than our separation factors.

Interestingly, the chiral selector does not contain any pi electron deficient functional group. The aromatic group in Fmoc lacks any electron deficient substituent and it has a conjugated aromatic unit. It is generally considered pi-electron rich when compared with unsubstituted benzene. Due to aromatic delocalization and the pi-electron donating properties of OH groups, 1,1′-bi-2-naphthol is also pi-electron rich compared with unsubstituted benzene. Therefore, pi–pi donor acceptor interaction cannot be the attractive interaction needed to achieve chiral recognition between the analyte and the chiral selector. In this example, such attractive interaction(s) are likely due to hydrogen bonding interactions.

4. Conclusions

Results of this study demonstrated the practical utility of the library screening method for chiral selector development. This example indicates that efficient chiral selectors could be developed using simple peptides without the pi-deficient 3,5-dinitrobenzoyl group. The batch screening method allows a large number of compounds to be studied quickly. Efficient chiral resolution of 1,1′-bi-2-naphthol is of practical importance. The current method to prepare enantiomerically pure 1,1′-bi-2-naphthol is rather inefficient, as it involves several steps. The excellent separation factors achieved in this pa-

per could enable the practical preparative chromatographic resolution of this useful racemic compound.

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