

Resolution of *tert*-Butyl-1-(2-Methylnaphthyl)phosphine Oxide Using Selectors Identified from a Chemical Combinatorial Library

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Resolution of racemic *tert*-butyl-1-(2-methylnaphthyl)-phosphine oxide **1, a chiral phosphorus compound, was achieved using selectors developed from a small peptide library. Separation factors as high as 3.2 were observed. The library consists of 81 peptide-based potential chiral selectors on polymeric synthesis resins. The linker needed to immobilize the identified chiral selectors onto silica gel proved important in the chiral separation; a longer linker provided a significantly higher separation factor in this study.**

Because human enzymes and cell surface receptors are all of chiral structures, enantiomers of a racemic compound may be absorbed, activated, and degraded by them in different manners. In some instances, this phenomenon causes two enantiomers of a racemic drug to have different or even opposite pharmacological activities. 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 enantioselective chromatography.¹

To develop efficient enantioselective stationary phases, several groups, including ours, have investigated the development of chiral selectors from chemical combinatorial libraries.² A number of approaches using either mixture combinatorial libraries or parallel combinatorial libraries (the high throughput method) have been investigated. These reported examples have demonstrated the feasibility of the mixture combinatorial library or the high-

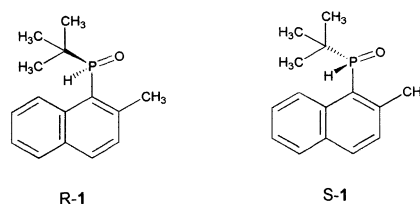


Figure 1. *R* and *S* enantiomers of racemic *tert*-butyl-1-(2-methylnaphthyl)phosphine oxide **1**.

throughput method for chiral selector development. However, in many of these reported examples, significant enantioselective separation of the chosen analytes or their close analogues had already been achieved prior to the combinatorial library studies. In this article, we would like to report an example for which the enantioselective separation of the analyte has not been well-studied previously.

The analyte of interest is *tert*-butyl-1-(2-methylnaphthyl)phosphine oxide **1** (Figure 1).³ In this compound, there are two chirogenic elements corresponding to the configurationally stable stereogenic phosphorus atom and the stereolabile aromatic-phosphorus rotation axis. At room temperature, because of the stereolabile aromatic-phosphorus rotation axis, oxide **1** exists as two instead of four diastereomers. Unlike compounds with chiral carbon atoms, far fewer synthetic methodologies are available to prepare enantiomerically pure compounds with chiral phosphorus atoms. Consequently, chromatographic resolution of such compounds takes on greater importance.⁴

EXPERIMENTAL SECTION

(For a list of the chemical abbreviations, see ref 5.)

General Supplies and Equipment. Solid-phase synthesis resins and amino acid derivatives were purchased from NovaBiochem (San Diego, CA). All other chemicals and solvents were purchased from either Aldrich (Milwaukee, WI), Fluka (Ronkonkoma, NY), or Fisher Scientific (Pittsburgh, PA). HPLC grade

(3) Gasparrini, F.; Lunazzi, L.; Mazzanti, A.; Pierini, M.; Pietrusiewicz, K. M.; Villani, C. *J. Am. Chem. Soc.* **2000**, *122*, 4776.

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(1) Stinson, S. C. *Chem. Eng. News* **1995**, *73* (41), 44–74.

(2) For examples, see the following papers and references cited within: (a) Welch, C. J.; Protopopova, M. N.; Bhat, G. *Enantiomer* **1998**, *3*, 471–476. (b) Lewandowski, K.; Murer, P.; Svec, F.; Frechet, J. M. J. *Chem. Commun.* **1998**, 2237. (c) Bluhm, L.; Wang, Y.; Li, T. *Anal. Chem.* **2000**, *72*, 5201–5205.

Dnb-[Asn, D-Asn, Gln, His, Hyp, Ser, Thr, Trp, Tyr]₂-Abu-AmPS

Figure 2. The 81-member library. Dnb: 3,5-dinitrobenzoyl; Hyp: *trans*-4-hydroxyproline; Abu: 4-aminobutyric acid ($\text{NH}_2(\text{CH}_2)_3\text{CO}_2\text{H}$); AmPS: aminomethylated polystyrene resin. Unless indicated otherwise, amino acids adopt the L configuration.

Allsphere silica gel (particle size, 5 μm ; pore size, 80 Å; surface area, 220 m^2/g) was purchased from Alltech (Deerfield, IL). 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) (EMerck, Merck KGaA, 64271 Darmstadt, Germany). Elemental analyses were conducted by Atlantic Microlab, Inc. (Norcross, GA). 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). The HI-TOP manual synthesizer required for parallel library synthesis was from Whatman Polyfiltronic (Rockland, Ma, USA).

Preparation of the Parallel 81-Member Library. The library (Figure 2) was synthesized using the polyfiltronic HI-TOP manual synthesizer. The experimental procedure for the synthesis of the Dnb-D-Asn-Thr member of the library is given below. Other library members were prepared following a similar sequence. To 75 mg (0.030 mmol in Abu) of Abu-AmPS resin⁶ in one well of the 96-well unfilter microplate, were added mixtures of Fmoc-Thr(tBu)-OH (16.4 mg, 0.0528 mmol), PyBOP (28.0 mg, 0.0528 mmol), and DIPEA (7.0 mg, 0.053 mmol) in 0.50 mL of DMF. After agitating for 2 h, the resins were filtered and washed with DMF, DCM, IPA, and DCM. The Fmoc protecting group was then removed by treatment with 0.60 mL of 20% piperidine in DMF for 20 min, followed by washing with DMF. Fmoc-D-Asn(Trt)-OH and dinitrobenzoic acid were then coupled to the resin following an identical reaction sequence. After that, the side chain protecting groups of Thr and D-Asn were removed by reacting with 0.6 mL of 95% TFA (2.5% water and 2.5% triisopropyl silane in TFA) for 1 h. The resin was then washed with DMF, DCM, IPA, and DCM, respectively, to yield the desired library member on the solid resin.

Screening of the Parallel Library with Chiral HPLC. To each well that contained resin was added racemic oxide **1** (0.15 mg) in CHCl_3 -heptane (2:8, 0.6 mL). After incubating the mixture for 3 h, the supernatants were filtered into a collection plate using the Polyfiltronic filtration manifold. The enantiomeric ratios of the supernatants were then analyzed by HPLC using a homemade Dnb-Leu column (column size, 50 \times 4.6 mm; mobile phase, 15% IPA/hexane; flow rate, 1.2 mL/min.; UV detection at 230 nm).

Preparation of Fmoc-Aminoundecanoic Acid (Fmoc-Aun-OH). To 11-aminoundecanoic acid (5.33 g, 26.5 mmol) and sodium

hydroxide (1.06 g, 26.5 mmol) in water (100 mL) was added gradually Fmoc-Osu (8.50 g, 25.2 mmol) in 100 mL of THF. After being stirred at room temperature for 4 h, the reaction mixture was adjusted to pH 1–3 by adding hydrochloric acid. The solution was extracted with ethyl acetate (3 \times 100 mL). The organic extracts were washed with water (3 \times 100 mL) and then dried over anhydrous sodium sulfate. Evaporation of the solvent yielded the crude product (9.5 g), which was recrystallized from ethyl acetate to give the pure product (9.3 g, 84%). ¹H NMR (CDCl_3): δ 12 (s, 1H), 7.8 (d, J = 7.2 Hz, 2H), 7.6 (d, J = 7.2 Hz, 2H), 7.2–7.4 (m, 5 H), 4.3 (d, J = 6.9, 2H), 4.2 (t, J = 6.9, 1H), 3.0 (t, J = 6 Hz, 2H), 2.2 (t, J = 6 Hz, 2H), 1.0–1.6 (m, 16H).

Preparation of Dnb-D-Asn-Thr-Aun-NH(CH₂)₃silica Stationary Phase. Other stationary phases were prepared by similar reactions. To Rink acid resin (3.00 g, 1.29 mmol/g) preswelled with DCM (20 mL, 30 min) were added Fmoc-Aun-OH (1.64 g, 3.9 mmol), DIC (0.49 g, 3.9 mmol), DMAP (157 mg, 1.29 mmol), and NMM (130 mg, 1.29 mmol) in DCM-DMF (2:1, 20 mL). After the mixture was agitated for 5 h, the resin was collected by filtration and washed with DMF, IPA, and DCM (20 mL \times 2).

The Fmoc group was then removed by treatment with 20% piperidine in DMF (20 mL) for 30 min. The deprotected Aun-ORink resin was collected and washed with DMF, IPA, and DCM (20 mL \times 2).

To Aun-ORink resin prepared above were added Fmoc-Thr(tBu)-OH (1.54 g, 3.9 mmol), PyBOP (2.0 g, 3.9 mmol), HOBt (174 mg, 1.29 mmol), and DIPEA (666 mg, 5.2 mmol) in DMF (20 mL). After agitating for 2 h, the resin was filtered and washed with DMF, IPA, and DCM (10 mL \times 2). The Fmoc group was then removed by the same procedures discussed in the previous paragraph.

The Thr(tBu)-Aun-ORink resin was then coupled with Fmoc-Asn(Trt)-OH by following exactly the same procedure as described above. After removing the Fmoc group, dinitrobenzoic acid was coupled to D-Asn(Trt)-Thr(tBu)-Aun-ORink resin. This coupling reaction was repeated one more time to ensure complete reaction. The reaction conditions are identical to those used for the coupling of Fmoc-Thr(tBu)-OH to the resin.

The resin was then treated with 1% TFA in DCM (20 mL, 10 min) to release Dnb-D-Asn(Trt)-Thr(tBu)-Aun-OH from the resin. The crude product obtained was purified by flash column chromatography on silica gel (mobile phase, 10% MeOH in DCM) to yield the desired Dnb-D-Asn(Trt)-Thr(tBu)-Aun-OH as a white solid (1.90 g, 100%). ¹H NMR: ($\text{DMSO}-d_6$) δ 0.98 (3H, d, J = 6 Hz), 1.05 (9H, s), 1.12–1.45 (16H, m), 2.1 (2H, t, J = 7.2 Hz), 2.8 (2H, m), 3.0 (2H, m), 3.9 (1H, q, J = 3 Hz), 4.1 (1H, dd, J = 6 Hz), 5.0 (1H, m), 7.1 (15H, s), 9.0 (1H, t, J = 2.1 Hz), 9.1 (2H, d, J = 2.1 Hz). ES-MS (m/z): 931.7 ($M + \text{Na}$)⁺.

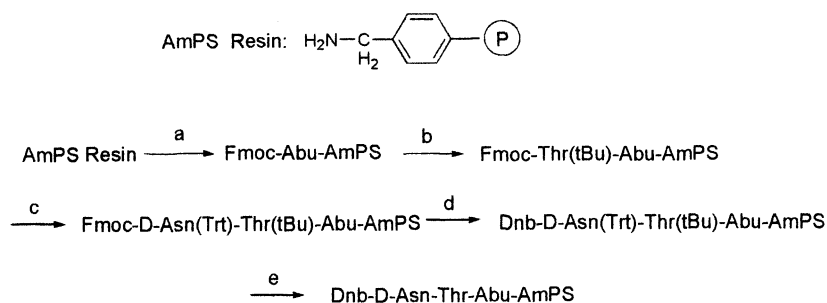
A mixture of Dnb-D-Asn(Trt)-Thr(tBu)-Aun-OH (581 mg, 0.64 mmol), PyBOP (333 mg, 0.64 mmol), HOBt (43 mg, 0.32 mmol), and DIPEA (124 mg, 0.96 mmol) in DMF (5 mL) was then added to aminopropyl silica gel (0.70 g, 0.32 mmol in amino group). After agitating for 3 h, the silica gel was collected by filtration and washed with DMF, IPA, and DCM (5 mL \times 2). The above coupling cycle was repeated one more time with Dnb-D-Asn(trt)-Thr(tBu)-Aun-OH (290 mg, 0.32 mmol), PyBOP (166 mg, 0.32 mmol), HOBt (22 mg, 0.16 mmol), and DIPEA (83 mg, 0.64 mmol). The unreacted free amine group on the silica gel was end-

(4) Absolute configuration of this compound was unknown at the time when this research was initiated but has been determined recently. See: Wang, F.; Wang, Y.; Polavarapu, P. L.; Li, T.; Drabowicz, J.; Pietrusiewicz, K. M.; Zygo, K. J. *J. Org. Chem.*, in press.

(5) Abu: 4-aminobutyric acid; Ahe: 6-aminoheptanoic acid; Aoc: 8-aminooctanoic acid; Aun: 11-aminoundecanoic acid; AmPS: aminomethylated polystyrene; NMM: *N*-methylmorpholine; PyBop: benzotriazolyl-oxo-tris[pyrrolidino]-phosphonium hexafluorophosphate; Fmoc: 9-fluorenylmethoxycarbonyl; DIC: diisopropylcarbodiimide; HOBt: 1-hydroxybenzotriazole; TFA: trifluoroacetic acid; DMAP: 4-(dimethylamino)pyridine; DIPEA: *N,N*-diisopropylethylamine; DCM: dichloromethane; DMF: dimethyl formamide; IPA: 2-propanol; Fmoc-Osu: 9-Fluorenylmethoxycarbonyl-hydroxysuccinimide; Dnb: 3,5-dinitrobenzoyl.

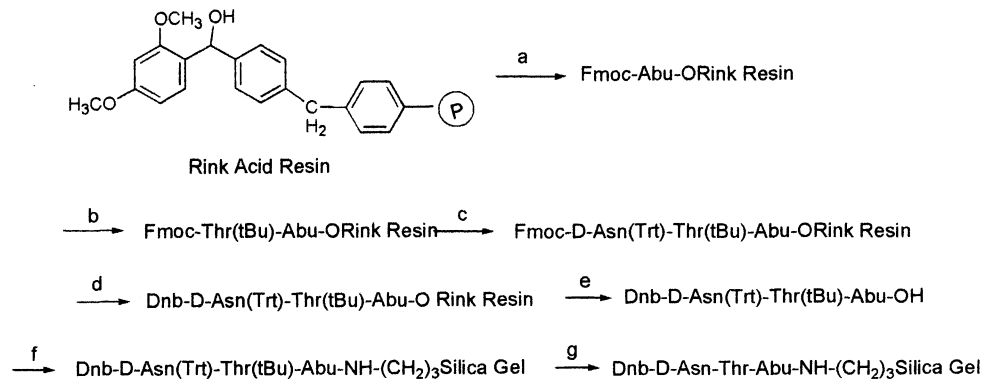
(6) Wang, Y.; Li, T. *Anal. Chem.* **1999**, *71*, 4178–4182.

Scheme 1. Preparation of the Dnb-D-Asn-Thr Member of the Parallel Library^a



^a Conditions: (a) Fmoc-Abu-OH, PyBop; (b) (1) piperidine, (2) Fmoc-Thr(tBu)-OH, PyBop; (c) (1) piperidine, (2) Fmoc-D-Asn(Trt)-OH, PyBop; (d) (1) piperidine, (2) Dnb-OH, PyBop; and (e) TFA.

Scheme 2. Synthesis of Dnb-D-Asn-Thr-Abu-(CH₂)₃-Silica Gel Stationary Phase



(a) Fmoc-Abu-OH, DIC; (b) (1) piperidine, (2) Fmoc-Thr(tBu)-OH, PyBop; (c) (1) piperidine, (2) Fmoc-D-Asn(Trt)-OH, PyBop; (d) (1) piperidine, (2) Dnb-OH, PyBop; (e) AcOH; (f) aminopropylsilica gel, PyBop; and (g) TFA.

capped by reacting with acetic anhydride (654 mg, 6.4 mmol) and pyridine (505 mg, 6.4 mmol) in 10 mL of DCM for 2 h. The silica gel was then collected by filtration. The protecting groups of Thr and D-Asn were then removed by reacting with 10 mL of 95% TFA (2.5% water and 2.5% triisopropyl silane in TFA) for 2 h. The desired chiral stationary phase was collected and washed with DCM, DMF, IPA, and methanol and then dried in vacuo. On the basis of elemental analysis,⁷ the surface ligand concentration was determined to be 0.17 mmol/g. The silical gel was packed into a 50 × 4.6 mm HPLC column.

Chromatographic Measurements. The Retention factor (*k*) equals $(t_r - t_0)/t_0$, in which *t_r* is the retention time and *t₀* is the dead time. Dead time *t₀* was measured with 1,3,5-*tert*-butylbenzene as the void volume marker according to the literature procedure.⁸ The dead time was measured to be 0.43 min with a flow rate of 1.2 mL/min for the column prepared above using CH₂-Cl₂/heptane (7/3) as the mobile phase.

RESULTS AND DISCUSSION

The library designed is a dipeptide library consisting of two amino acid modules (Figure 2). The two modules are identical, both contain the same nine amino acids. All of the possible combinations of two modules yield a library containing a total of 81 members. The amino acids in the peptide module are chosen on the basis of consideration of the molecular features of the target analyte. The analyte contains a flat, aromatic naphthalene ring in addition to a hydrogen bond acceptor group. The amino acids chosen contain side chains that could interact with the analyte through either hydrogen bonding interaction (Asn, D-Asn, Hyp,

Ser, Thr), aromatic stacking (Trp), or a combination of both (His, Tyr). Structural diversity is achieved by the application of amino acids with side chains that are significantly different from each other, including a D-amino acid.⁹ In addition, the use of the electron deficient dinitrobenzoyl (Dnb) group as the N-terminal end-capping group of the peptides provide another potential interaction site with the analyte.¹⁰

The resin chosen for the synthesis of this library was aminomethylated polystyrene (AmPS) resin, a derivative of the widely used Merrifield resin. Unlike silica gel, such a resin allows the reliable synthesis of the library. The synthesis of this library was performed conveniently using a Hi-top filter plate manual synthesizer.¹¹ In this synthesizer, resin can be added to the wells of a 96-well filter plate. By adding individual amino acids into each well, 96 different peptides can be synthesized in one run. This Hi-top system allows for quick filtration between each synthetic step without the need to remove any resin from the plate, and the overall efficiency of this synthetic process is very high. In terms of the chemistry involved, Fmoc solid phase synthesis was chosen, and the detailed chemistry is illustrated in Scheme 2 with the synthesis of the Dnb-D-Asn-Thr member of the library. In this synthesis, commercially available, side-chain-protected forms of Thr, the *N*-α-Fmoc-O-*tert*-butyl-L-threonine (Fmoc-Thr(tBu)-OH)

(7) Yang, A.; Li, T. *Anal. Chem.* **1998**, *70*, 2827–2830.

(8) Pirkle, W. H.; Welch, C. J. *J. Liq. Chromatogr. A* **1991**, *14*, 1–8.

(9) Billiot, E.; Warner, I. M. *Anal. Chem.* **2000**, *72*, 1740–1748.

(10) (a) Pirkle, W. H. *Chirality* **1997**, *9*, 103–104. (b) Wainer, I. W.; Caldwell, J. *Chirality* **1997**, *9*, 95–96.

(11) The Hi-top system, Polyfiltronics, 100 Weymouth Street, Rockland, MA, 02370.

Table 1. Ratios of Peak Heights Obtained for the Supernatants for the 81 Members of the Library^a

His-His 1.168	D-Asn-His 1.292	Hyp-His 1.135	Trp-His 1.191	Ser-His 1.218	Thr-His 1.279	Asn-His 1.121	Gln-His 1.152	Tyr-His 1.137
His-D-Asn 1.135	D-Asn-D-Asn 1.489	Hyp-D-Asn 1.145	Trp-D-Asn 1.145	Ser-D-Asn 1.203	Thr-D-Asn 1.371	Asn-D-Asn 1.065	Gln-D-Asn 1.224	Tyr-D-Asn 1.131
His-Hyp 1.157	D-Asn-Hyp 1.212	Hyp-Hyp 1.149	Trp-Hyp 1.209	Ser-Hyp 1.230	Thr-Hyp 1.334	Asn-Hyp 1.081	Gln-Hyp 1.177	Tyr-Hyp 1.173
His-Trp 1.155	D-Asn-Trp 1.506	Hyp-Trp 1.177	Trp-Trp 1.177	Ser-Trp 1.269	Thr-Trp 1.300	Asn-Trp 0.968	Gln-Trp 1.151	Tyr-Trp 1.143
His-Ser 1.128	D-Asn-Ser 1.380	Hyp-Ser 1.142	Trp-Ser 1.122	Ser-Ser 1.221	Thr-Ser 1.308	Asn-Ser 1.047	Gln-Ser 1.162	Tyr-Ser 1.128
His-Thr 1.158	D-Asn-Thr 1.718	Hyp-Thr 1.135	Trp-Thr 1.152	Ser-Thr 1.197	Thr-Thr 1.269	Asn-Thr 1.014	Gln-Thr 1.151	Tyr-Thr 1.096
His-Asn 1.162	D-Asn-Asn 1.329	Hyp-Asn 1.142	Trp-Asn 1.129	Ser-Asn 1.303	Thr-Asn 1.162	Asn-Asn 0.976	Gln-Asn 1.091	Tyr-Asn 0.997
His-Gln 1.200	D-Asn-Gln 1.326	Hyp-Gln 1.155	Trp-Gln 1.219	Ser-Gln 1.259	Thr-Gln 1.303	Asn-Gln 1.085	Gln-Gln 1.166	Tyr-Gln 1.201
His-Tyr 1.131	D-Asn-Tyr 1.240	Hyp-Tyr 1.124	Trp-Tyr 1.154	Ser-Tyr 1.252	Thr-Tyr 1.365	Asn-Tyr 1.021	Gln-Tyr 1.115	Tyr-Tyr 1.155

^a Ratio of the peak heights of racemic mixture **1** is 1.193. Library template, Dnb-Aa1-Aa2-Abu-AmPS.

and of D-Asn, *N*- α -Fmoc-*N*- β -trityl-D-asparagine (Fmoc-D-Asn(Trt)-OH) are needed. Other library members were synthesized following similar reactions.

For screening purpose, an equal amount of the racemic analyte (0.15 mg, 0.00061 mmol) in a mixture of chloroform and heptane (2:8, 0.60 mL) was added to each of the wells that contained 0.03 mmol of selector. After equilibration, the supernatants were collected into a collection plate. Enantiomeric ratio of the supernatant was analyzed using a Dnb-Leu column prepared previously in the laboratory, which yielded a separation factor of 1.2. The ratio of the two peak heights was used as the relative measure of the enantiomeric purity. For the racemic analyte **1**, the ratio was measured to be 1.193. Ratios of peak heights obtained for the supernatants for all 81 wells of the library are summarized in Table 1.

As seen from Table 1, several promising leads were identified. Some of the ratios are higher than the ratio for racemic analyte and some others are lower, indicating selectors with opposite selectivities exist in the library. It is also noted that several promising leads contain D-Asn, which has been investigated and used previously in chiral micellar electrophoresis studies.⁹ The ratio of the peak heights for D-Asn-D-Asn is 1.489, 25% higher than that of the racemic mixture; In contrast, the ratio of the peak heights for Asn-Asn is 0.976, 22% lower than that of the racemic mixture. These two results demonstrate the reliability of the screening method.

The first attempt was to immobilize the most promising selector D-Asn-Thr, which corresponds to an enantiomer ratio of 1.44 as calculated from its peak height ratio in Table 1, using a short Abu linker. Immobilization of this selector onto silica gel was achieved according to the chemistry described in Scheme 2. The key in this synthetic scheme was the use of an acid-sensitive resin (Rink acid resin) to prepare a peptide acid fragment in which the side chains of Thr and D-Asn are still protected. The protected peptide acid fragment was then coupled to aminopropylsilica gel. After coupling to the solid support, the protecting groups of Thr and D-Asn were removed by treatment with TFA, yielding the desired stationary phase on the solid support. The resulting

Table 2. Chiral Resolution of Racemic *tert*-Butyl-1-(2-methylnaphthyl)phosphine Oxide **1**

CSPs	linker length	k_r^a	α	surface loading (mmol/g)
Dnb-D-Asn-Thr-Abu-NH(CH ₂) ₃ silica	(CH ₂) ₃	58	1.02	0.16
Dnb-D-Asn-Thr-Ahe-NH(CH ₂) ₃ silica	(CH ₂) ₅	3.8	1.9	0.17
Dnb-D-Asn-Thr-Aoc-NH(CH ₂) ₃ silica	(CH ₂) ₇	1.8	2.3	0.17
Dnb-D-Asn-Thr-Aun-NH(CH ₂) ₃ silica	(CH ₂) ₁₀	5.9	3.2	0.18
Dnb-Asn-Asn-Aun-NH(CH ₂) ₃ silica	(CH ₂) ₁₀	3.2	1.7	0.18

^a k_r is the retention factor of the least retained enantiomer. Mobile phase, 7/3 CH₂Cl₂/heptane.

stationary phase was then packed into a column using a standard slurry packing method.¹²

Surprisingly, the resulting stationary phase provided a separation factor of only 1.02. The lack of correlation could be attributed to the differences of the solid supports used in these experiments. For resin equilibration experiments, the selector was attached to a polymer resin suitable for solid-phase synthesis, whereas chromatographic experiments were performed on silica gel. Unlike polymeric resin, silica gel support is hydrophilic and is covered with silanol groups. Potential nonspecific interaction of these surface polar silanol groups with the analyte could lead to a reduction in chiral selectivity.

We then immobilized the selector, using longer linkers, hoping that the distance of separation between the chiral selector and silica gel surface would minimize the impact of the polar surface. Three new stationary phases were prepared, one with an amino-hexanoic acid (Ahe) linker, one with an aminooctanoic acid (Aoc) linker, and one with an aminoundecanoic (Aun) acid linker. They were prepared according to essentially the same procedure as described for the immobilization of selector with the short Abu linker (Scheme 2).

Separation factors obtained for these stationary phases are summarized in Table 2. Excellent resolution of the phosphorus

(12) Poole, C. F.; Poole, S. K. *Chromatography today*; Elsevier: New York, 1991; pp 350–353.

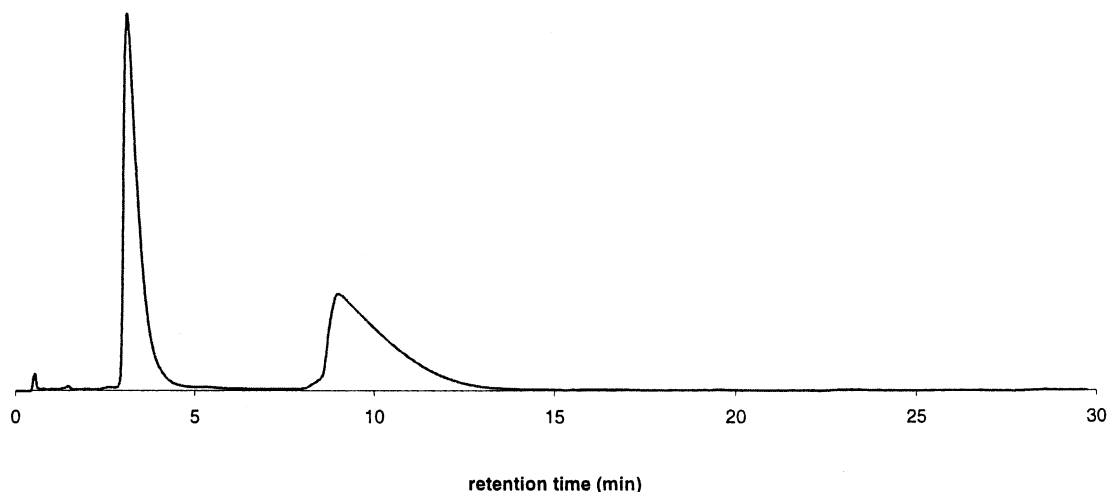


Figure 3. Chiral resolution of *tert*-butyl-1-(2-methylnaphthyl)phosphine oxide **1** with Dnb-D-Asn-Thr-Aun-NH(CH₂)₃silica. First eluting peak was assigned an *R* configuration.⁴

analyte was achieved when aminoundecanoic acid was used as the linker (Figure 3). Dependence of the separation factors on linker length is apparent from Table 2.

Another selector, Dnb-Asn-Asn, which yields the opposite selectivity as the D-Asn-Thr selector and which corresponds to an enantiomer ratio of 0.82 as calculated from its peak height ratio in Table 1, was subsequently immobilized with the aminoundecanoic acid linker. Excellent resolution of the target racemic analyte was also achieved with this selector. As expected, the opposite elution order was observed with this stationary phase. One can, of course, prepare a stationary phase using the opposite enantiomer of D-Asn-Thr, Asn-D-Thr to provide such a reverse elution order.

CONCLUSIONS

Results of this study demonstrated the practical potential of the library screening method using synthesis resins. Unlike analytes used for our previous model studies, this target analyte has not been well-studied previously in chiral separation. In addition, resolution of chiral phosphorus compounds are of special interest, because far fewer synthetic methods are capable of preparing this class of compounds in enantiomerically pure form. The excellent separation factors achieved enable the preparative resolution of this racemic compound.

It should be pointed out that discrepancies between the outcomes of the screening experiment and the subsequent

chromatographic experiments are possible, because the chemical properties of the synthesis resins are different from the chromatographic resins. However, a longer linker could be used to minimize the impact of solid support in order to maintain good correlation. Other approaches to avoid the possible discrepancy are being explored. Such a difference in the properties of the solid supports in these two types of experiments also prevents a quantitative correlation at this time. With improved surface modification procedure or different support for synthesis or for chromatography, such a correlation may become feasible in the future.

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