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Polysodium *N*-Undecanoyl-L-leucylvalinate: A Versatile Chiral Selector for Micellar Electrokinetic Chromatography

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Dipeptide micelle polymers are a new class of polymeric surfactants of which the polysodium undecanoyl-L-leucylvalinate (poly-L-SULV) was found to be a broadly applicable chiral selector for micellar electrokinetic chromatography. This negatively charged dipeptide micelle polymer is a high molecular weight compound with large countercurrent mobility, zero critical micelle concentration, low aggregation number, and high solubility in water or water−organic solvents. In an extensive chiral screening program, enantioseparation of 75 racemic compounds was tested with poly-L-SULV as chiral pseudostationary phase in neutral pH and basic pH background electrolytes. A total of 58 out of 75 racemic compounds could be resolved after choosing an appropriate concentration of poly-L-SULV. Although anionic chiral analytes are difficult to resolve using poly-L-SULV, the percent success rate for chiral resolution of cationic (77%) and neutral (85%) racemates was very high. Aspects regarding electrostatic, steric, hydrophobic, and hydrogen-bonding interactions of this dipeptide micelle polymer with various classes of chiral analytes are discussed.

Molecular micelles (aka polymeric surfactants or micelle polymers) have recently received considerable attention as stable pseudostationary phases for achiral[1−6] and chiral separations[7−11] in micellar electrokinetic chromatography (MEKC). The use of molecular micelles in capillary electrophoresis (CE) appears to offer distinct advantages over conventional surfactant micelles. Unlike conventional micelles, polymeric surfactants can be purified and can be used at concentrations below the critical micelle concentration (cmc) because they are covalently linked. This, in turn, provides much higher efficiencies, relatively lower Joule heating, and rapid analysis in CE as compared to equivalent concentrations of monomeric surfactant. Another very important advantage of polymeric surfactants is that, after the separation of charged and polar compounds, sensitive detection of such compounds can be achieved using more MS-friendly conditions[12−15] than those required using cyclodextrins[16,17] or conventional micelles.[18,19]

Dipeptide micelle polymers belong to a relatively new class of chiral selectors for MEKC. These dipeptide polymers were introduced by Shamsi and co-workers in 1997.[20] Since then, various dipeptide micelle polymers with different chiral centers,[21,22] chiral combinations,[23−25] and configurations[26] have been synthesized and evaluated. On the basis of information derived in several of our papers, we have attempted to design a unique dipeptide surfactant for general chiral separations. So far, it appears that the most versatile chiral dipeptide surfactant is polysodium *N*-undecanoyl-L-leucylvalinate (poly-L-SULV). Table 1 provides the chemical structure and physicochemical properties of the chiral

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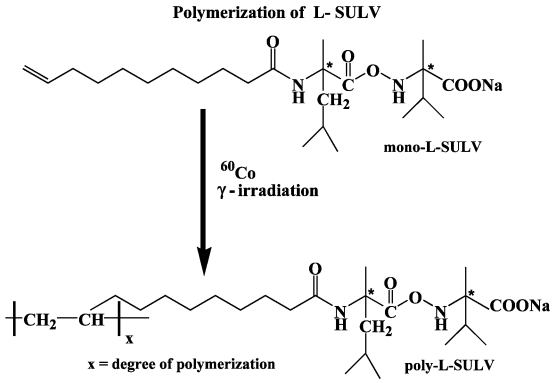
Table 1. Physicochemical Properties of Sodium *N*-Undecenoyl-L-leucylvalinate (L-SULV) and Polysodium *N*-Undecanoyl-L-leucylvalinate (Poly-L-SULV)

Characteristic	L-SULV	poly-L-SULV
molecular weight	418.6	14,650 (± 625)[a]
critical micelle concentration (CMC) [mM][b]	7	0
optical rotation	-0.39	-0.39
effective electrophoretic mobility (cm^2V^{-1}/s^{-1})	-3.74 X $10^{-4(c)}$ -4.19 X $10^{-4(d)}$	-3.76 X $10^{-4(c)}$ -4.65 X $10^{-4(d)}$ -3.89 X $10^{-4(e)}$
Aggregation number[b]	39	18
Partial specific volume[Vbar (mL/g)]	0.83[d]	0.80[d]



[a] Obtained from ref 27. [b] Obtained from ref 28. [c] Determined using 20 mM borate buffer, 20 mM (unpolymerized L-SULV or poly-L-SULV at pH 9.3, +30 kV using pyrene as a micelle marker at 254 nm. [d] Determined using 25 mM borate buffer, 20 mM (unpolymerized L-SULV or poly-L-SULV at pH 9.3, + 30 kV using *tert*-butylanthracene as a micelle marker at 254 nm. [e] Determined using 20 mM borate buffer with direct injection of polymer solution at +30 kV.

surfactant, L-SULV, and the respective polymer, poly-L-SULV. As noted in Table 1, the monomer of L-SULV contains two chiral centers of the same L-configuration with N-terminal leucine and C-terminal valine amino acids attached to a polymerizable C_{11} hydrocarbon chain. Comparison of the physicochemical properties of the monomers and polymers of L-SULV reveals that the latter is a high molecular weight, highly charged species with higher electrophoretic mobility. Although the partial specific volume and electrophoretic mobility of unpolymerized L-SULV and poly-L-SULV are very similar, poly-L-SULV has a significantly lower aggregation number than L-SULV, as well as a zero cmc.

In this work, chiral recognition properties of poly-L-SULV were investigated using 75 racemic compounds with various structural features. Chiral MEKC parameters (e.g., pH, type and concentration of background electrolyte, and concentration of micelle polymers) were optimized for each class of compounds. The enantioseparation of various classes of cationic, anionic, and neutral analytes is correlated with their stereoselective interaction with poly-L-SULV. Our major interest in these studies aims at finding reliable rules that affect the chiral recognition of various enantiomers using poly-L-SULV as a chiral pseudostationary phase.

EXPERIMENTAL SECTION

Materials. The analytes were obtained either as racemic mixtures or pure enantiomers from Sigma Chemical Co (St. Louis, MO) or the Aldrich Chemical Co. (Milwaukee, WI). The background electrolytes (BGEs) such as sodium phosphate monobasic, sodium phosphate dibasic, boric acid, tris(hydroxymethyl)aminomethane (TRIS), and sodium borate and cyclohexylaminopropanesulfonate (CAPS) were of analytical reagent grades and were obtained from Sigma. Chemicals used for the synthesis of surfactants included the following: *N*,*N*′-dicyclohexylcarobodiimide (DCC), *N*-hydroxysuccinimide, and undecylenic acids were also obtained from Sigma. The dipeptide L-leucylvalinate was purchased from BaChem Bioscience Inc (King of Prussia, PA) and was used as received.

Instrumentation. All enantioseparations were performed using an Agilent CE system. This CE instrument was equipped with (1) 0−30-kV high-voltage built-in power supply, (2) a diode array detector for UV detection, and (3) Chemstation software for system control and data handling. The fused-silica capillaries (50-μm i.d., 320-μm o.d.) obtained from Polymicro Technologies (Phoenix, AZ) were employed in all experiments. The effective length of the capillaries was 56 cm; the total length was 64.5 cm.

Synthesis of Polysodium *N*-Undecanoyl-L-leucylvalinate. The molecular structure of the polymeric dipeptide surfactant poly-L-SULV is shown in Table 1. This dipeptide micelle polymer was synthesized from enantiomerically pure L-leucylvalinate using a procedure previously reported elsewhere.[20,21]

Determination of Partial Specific Volume. Partial specific volume (*V*) is defined as the increase in volume when 1 g of dry solute is dissolved in a large volume of solvent. This parameter is used to estimate the approximate volume of a particle since the exact volume of a particle is difficult to measure. A high-precision densitometer (model DMA58) from Anton Paar USA (League City, TX), was used to determine density measurements. Air and water were used for calibration. The partial specific volume measurements were performed as described in earlier publications from our group.[10,27] In brief, five solutions containing 0, 5, 10, 15, 20, and 25 mg of pseudostationary phase were dissolved in 50 g of solvent (100 mM NaCl). The *V* values were determined by plotting the inverse of the density ($1/\rho$) of the solutions as a function of the weight fraction (*W*) of the chiral pseudostationary phase according to the following equation:

$$1/\rho = V + W\partial((1/\rho)/\partial W) \qquad (1)$$

Preparation of MCE Buffers and Analyte Solutions. The background electrolytes at pH 6.5 or 7.0 were prepared by mixing 25 mM solutions of mono and dibasic sodium phosphate or 30 mM dibasic sodium phosphate and 275 mM boric acid. The BGE at pH 8.5 was 25 mM TRIS/sodium borate or 300 mM CAPS/50 mM borate. The basic BGEs at pH 10.2 and 11.2 were prepared using either 25 mM TRIS/dibasic sodium phosphate or 50 mM dibasic sodium phosphate. In all cases, the desired pH values of the BGEs were achieved by using either 1 M NaOH or 1 M phosphoric acid. After the pH was adjusted, various equivalent monomer concentrations (EMC) of the poly L-SULV surfactant were added to the BGE solutions. Finally, the BGEs containing

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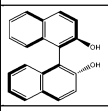
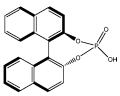
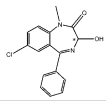
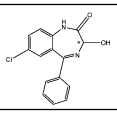
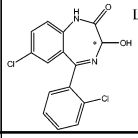
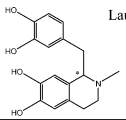
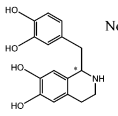
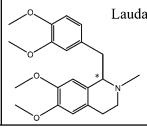
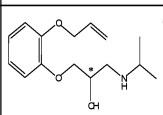
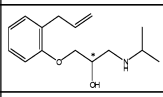
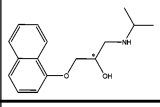
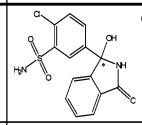
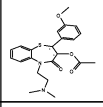
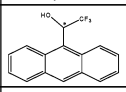
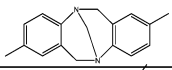
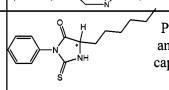
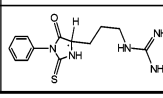
Table 2. Class I: Examples of Enantiomers That Exhibit Strong Chiral Interactions with Poly-L-SULV. Enantiomeric Resolution (R_s), Capacity Factors (k_2'), and Selectivity Factors (α) of Enantiomers Separated with 5−20 mM Poly-SULV

Charge of compound under given conditions	Compound	concentration of poly-L-SULV (mM)	Rs	k_2'	$\alpha^{(i)}$
1. Partially anionic	 (±)-1,1'-bi-2-naphthol[a]	5	2.05	1.54	1.034 (±0.003)
2. Neutral	 (R,S)-1,1'-binaphthyl-2,2'-diamine[a]	5	1.74	1.74	1.023 (±0.005)
3. Anionic	 (±)-binaphthyl-2,2'-diyl-hydrogen-phosphate[a]	7	1.52	1.22	1.015 (±0.002)
4. Neutral	 Temazepam[b]	12	3.43	0.85	1.023 (±0.006)
5. Neutral	 Oxazepam[b]	12	1.70	1.25	1.011 (±0.009)
6. Neutral	 Lorazepam[b]	12	2.53	1.44	1.013 (±0.008)
7.Cationic	 Laudanosoline[c]	20	2.36	1.14	1.044 (±0.004)
8. Cationic	 Norlaudanosoline[c]	10	4.27	1.37	1.072 (±0.007)
9. Cationic	 Laudanosine[c]	20	1.44	1.35	1.014 (±0.005)

Charge of compound under given conditions	Compound	concentration of poly-L-SULV (mM)	Rs	k_2'	$\alpha^{(i)}$
10. Partially Cationic	 Oxprenolol[d]	12.5	1.41	0.65	1.014 (±0.006)
11.Partially Cationic	 Alprenolol[e]	12.5	1.52	0.82	1.016 (±0.003)
12.Partially Cationic	 Propranolol[e]	12.5	1.41	1.32	1.015 (±0.005)
13. Neutral	 Chlorothalidone[f]	6	1.84	2.56	1.032 (±0.003)
14. Neutral	 Diltiazem[g]	6	1.12	4.22	1.021 (±0.006)
15. Neutral	 2,2,2-Trifluoro-anthrylethanol[g]	6	3.14	2.57	1.044 (±0.007)
16. Neutral	 Troger's Base[f]	5	2.20	1.67	1.026 (±0.005)
17. Neutral	 PTH-α-amino-caprylic acid[h]	10	2.66	4.23	1.082 (±0.006)
18. Cationic	 PTH-Arginine[h]	10	1.55	4.55	1.056 (±0.008)

[a] 100 mM Tris/10 mM borate buffer, pH 10.2, 90 mbar s, +30 kV, 220 nm. [b] 25 mM Tris/25 mM borate buffer, pH 8.5, 30 mbar s, +30 kV, 220 nm. [c] 50 mM phosphate buffer, pH 7, 20 mbar s, +30 kV, 220 nm. [d] 50 mM borate buffer, pH 9.2, 5 mbar s, +30 kV, 220 nm. [e] 300 mM CAPS, 50 mM borate, pH 8.5, 5 mbar s, 220 nm. [f] 30 mM phosphate buffer, pH 7.0, 12 °C, 30 mbar s, +30 kV. [g] 50 mM borate buffer, pH 11.2, 12 °C, 30 mbar s, +30 kV, 254 and 280 nm. [h] 275 mM boric acid/30 mM dibasic phosphate, 10 mM triethylamine, 50 mbar s, +30 kV, 269 nm. [i] Confidence interval at 90% was calculated for four consecutive injections of each analyte.

poly-L-SULV were filtered through a 0.45-μm nylon syringe filter (Nalgene, Rochester, NY) by creating a vacuum inside the syringe. This was followed by ultrasonication for 10 min to ensure properly degassed micellar running buffers. The analytes were dissolved either in 50:50 methanol/water or 80:20 methanol/water to give a final concentration of 0.2, 0.5, or 1 mg/mL.

Capillary Electrophoresis Procedure. New capillaries were washed with 1 N NaOH for 1 h at 60 °C, followed by a 10-min rinse with triply distilled water. Between injections, the capillary was flushed for 2 min each with water and micellar buffer. The separation parameters such as retention factor (k'), enantioselective factor (α), and resolution factor (R_s) were calculated using the equations described elsewhere.[9,20]

RESULTS AND DISCUSSION

Various experimental factors such as pH, concentration and type of BGE, concentration of poly-L-SULV, sample concentration, and injection size were optimized for the screening of 75 racemic compounds. Although a complete optimization of the aforementioned experimental factors was independently conducted for each racemate, the concentration of poly-L-SULV served as the basis for the analytes' classification. Therefore, in this study, analyte enantiomers were classified into three groups according to the concentration range of poly-L-SULV required to provide enantioseparation.

Enantioseparation of Class I Analytes. As shown in Table 2, a total of 18 racemic compounds, (i.e., 24% of all analytes

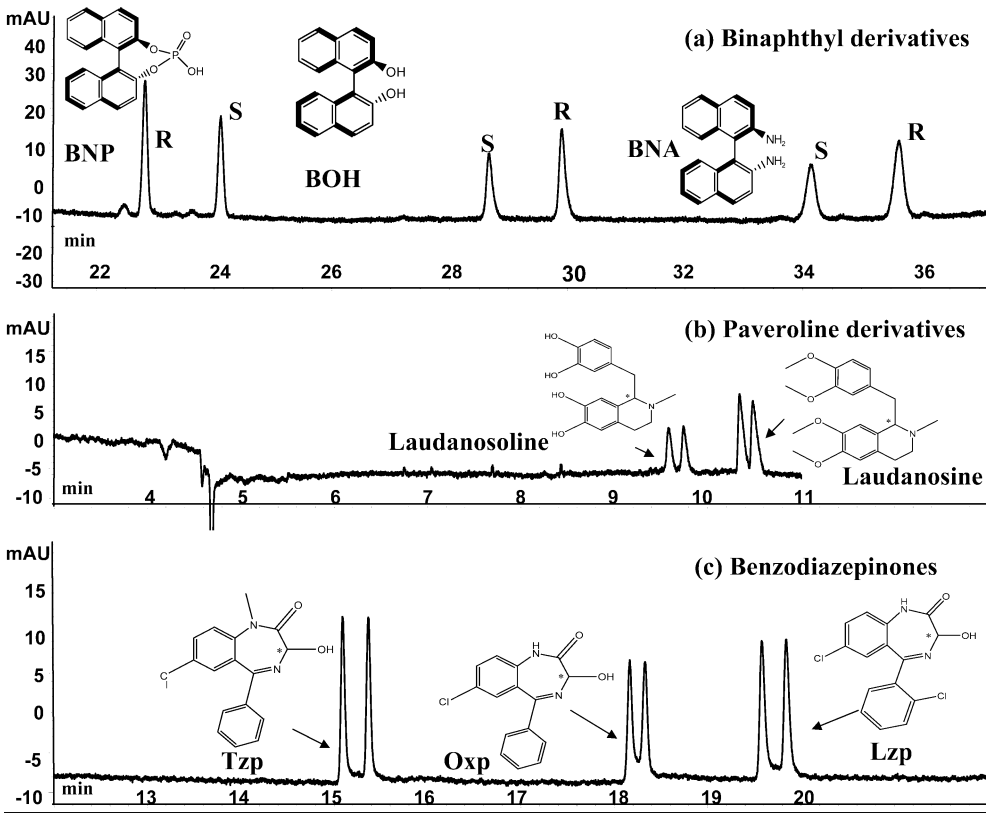


Figure 1. (a) Simultaneous separation and enantioseparation of binaphthyl derivatives. MEKC conditions: 15 mM poly-L-SULV, 14 °C, pH 10.0, 100 mM Tris/10 mM borate buffer. Peak Identification: 0.2 mg/mL BNP = 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate, BOH = 1,1′-bi-2-naphthol, BNA = 1,1′-bi-2-naphthylamine. Pressure injection: 90 mbar·s; +30 kV applied for separation; UV detection at 220 nm. (b) Simultaneous separation and enantioseparation of paveroline derivatives. MEKC conditions: 20 mM poly-SULV, pH 7.0, 50 mM phosphate buffer. (c) Simultaneous separation and enantioseparation of benzodiazepinones. MEKC conditions: 25 mM poly-SULV, pH 8.5, 100 mM Tris/10 mM borate buffer. Other conditions in (b) and (c) are the same as in (a).

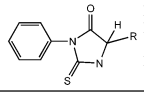
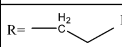
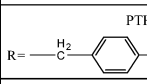
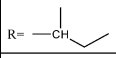
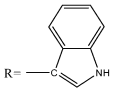
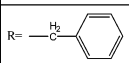
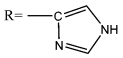
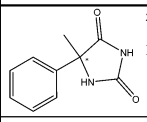
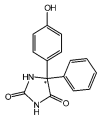
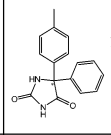
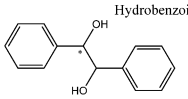
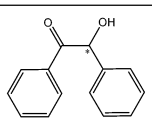
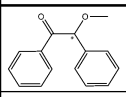
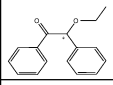
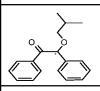
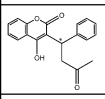
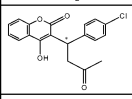
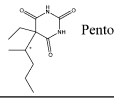
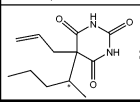
investigated) could be resolved with R_s values ranging from 1.12 to 4.27. Such racemic compounds can be classified as class I analytes (i.e., those exhibiting strong chiral interactions) because very low concentrations (between 5 and 20 mM) of poly-L-SULV are needed for the chiral separation of these compounds. This class includes binaphthyl derivatives, benzodiazepinones, paveroline derivatives, several members of the β-blocker family, and a variety of other classes of cationic and neutral compounds.

Racemates of neutral (e.g., 1,1′-binaphthylamine) and partially anionic (e.g., 1,1′-bi-2-naphthol) binaphthyl derivatives are particularly easy to resolve and showed baseline resolution at concentrations as low as 5 mM poly-L-SULV. In contrast, chiral separation of the anionic binaphthyl derivative, 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate, requires a slightly higher concentration, i.e., 7 mM poly-L-SULV (Table 2). However, simultaneous enantioseparation of all three binaphthyl derivatives requires at least 15 mM poly-L-SULV (Figure 1a). This is not so surprising since an increase in polymeric surfactant concentration essentially widens the chiral elution window.

Simultaneous chiral resolution of the paveroline derivative (i.e., a cationic biosynthetic precursor of morphine) and one of its analogues (laudanosoline) was achieved using 20 mM poly-L-SULV (Figure 1b). Comparison of the migration times and enantioresolution of laudanosoline and laudanosine demonstrates the ability of poly-L-SULV to discriminate not only on the basis of hydrophobicity but also on the basis of hydrogen-bonding interactions. The hydrophobically driven complexation between poly-L-SULV and laudanosine resulted in weaker chiral interaction than the hydrogen-bonding interactions offered by the multiple hydroxy groups found on laudanosoline or norlaudanosoline (Table 2). Moreover, norlaudanosoline possesses a secondary amine group that offers an additional site for hydrogen bonding, producing much greater enantioresolution than that observed for either laudanosine or laudanosoline.

Another interesting comparison of class I analytes can be made for the chiral separation of the benzodiazepinones. The three racemates of benzodiazepinones separated in Figure 1c possess a similar aromatic skeleton. The differences lie in the number and type of functional groups attached to the aromatic ring. In the case of temazepam, an extra methyl group is located at the nitrogen in the seven-member ring, whereas an additional chloro group is located in the ortho position of the lower benzene ring of lorazepam. As shown in Table 2, poly-L-SULV resolved enantiomers of temazepam, oxazepam, and lorazepam with resolution values of 3.44, 1.70, and 2.53, respectively. Surprisingly, temazepam, which has fewer hydrogen-bonding sites than oxazepam or lorazepam, provides faster separation with greater enantioresolution. Evidently, the hydrogen-bonding moiety at the nitrogen of the seven-member ring of benzodiazepinones seems to play a minor role in chiral recognition since the presence of a methyl group at the nitrogen in the seven-member ring of temazepam does not sterically inhibit chiral recognition. Since the chiral resolution and chiral selectivity values of benzodiazepinones (temazepam, oxazepam, lorazepam) do not follow any particular

Table 3. Class II: Examples of Enantiomers That Exhibit Moderate Chiral Interactions with Poly-L-SULV. Enantiomeric Resolution (R_s), Capacity Factors (k'), and Selectivity Factors (α) of Enantiomers Separated with 30−50 mM Poly-SULV

Charge of compound under given conditions	Compound	concentration of poly-L-SULV (mM)	Rs	k_2'	$\alpha^{(d)}$
	 Phenylthio-hydantoin (PTH) R = amino acid chain				
1. Neutral	 PTH-valine[a]	50	2.33	0.94	1.062 (±0.002)
2. Neutral	 PTH-norvaline[a]	50	4.92	1.23	1.085 (±0.003)
3. Neutal	 PTH- tyrosine[a]	50	1.46	2.23	1.033 (±0.007)
4. Neutral	PTH-norleucine[a]	50	2.82	2.47	1.056 (±0.008)
5. Neutral	 PTH-isoleucine[a]	50	3.02	1.56	1.063 (±0.002)
6. Neutral	 PTH-tryptophan[a]	50	1.84	2.54	1.042 (±0.006)
7. Neutral	 PTH-phenylalanine[a]	50	2.63	2.25	1.046 (±0.007)
8.Cationic	 PTH-histidine[a]	50	1.47	1.43	1.035 (±0.006)
9. Neutral	 5-methyl-5-phenyl-hydantoin[a]	30	1.44	1.26	1.016 (±0.004)
10. Neutral	 5-(4-hydroxy phenyl)-5-phenylhydantoin[a]	30	2.23	1.56	1.053 (±0.002)
11. Neutral	 5-(4-methyl-phenyl)-5-phenylhydantoin[a]	30	1.86	2.17	1.032 (±0.006)
12. Neutral	 Hydrobenzoin[b]	50	1.45	1.72	1.017 (±0.002)
13. Neutral	 Benzoin[b]	50	2.95	2.32	1.054 (±0.006)
14. Neutral	 Benzoin methylether [b]	50	2.33	3.56	1.056 (±0.007)
15. Neutral	 Benzoin ethylether [b]	50	0.62	3.82	1.017 (±0.005)
16. Neutral	 Benzoin isobutyl-ether [b]	50	0.30	4.22	1.011 (±0.005)
17. Anionic	 Warfarin[a]	50	1.51	0.88	1.023 (±0.005)
18. Anionic	 Coumachlor[a]	50	3.12	1.21	1.038 (±0.002)
19. Partially Anionic	 Pentobarbital[c]	50	1.48	1.33	1.014 (±0.003)
20. Partially Anionic	 Secobarbital[c]	50	1.52	1.60	1.018 (±0.003)

[a] 275 mM boric acid, 30 mM Na_2HPO_4, 10 mM triethylamine, pH 7.2, 30−150 mbar s, 269 nm. [b] 50 mM phosphate buffer, pH 7.0, 12 °C, +30 kV, 220 nm. [c] 50 mM phosphate buffer, pH 7.2, 12 °C, +30 kV, 220 nm. [d] Confidence interval at 90% was calculated for four consecutive injections of each analyte.

trends, no clear explanation is available at this time for the preferential sites of interaction of these analytes with poly-L-SULV.

In general, the enantioselectivity of poly-L-SULV for the separation of class I analytes seems to be similar to single amino acid surfactants such as polysodium *N*-undecanoylleucinate (poly-L-SUL) and polysodium *N*-undecanoylvalinate (poly-L-SUV).[25,26] However, some chiral anionic compounds (e.g., 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate) and neutral compounds (e.g., oxazepam) can be resolved with poly-L-SULV that could not be separated (under neutral to basic conditions) using either poly-L-SUV or poly-L-SUL alone (data not shown). In addition, for all concentrations of poly-L-SULV, the separation times for the enantiomeric resolution of cationic compounds are significantly lower than those obtained using either poly-L-SUV or poly-L-SUL.

Enantioseparation of Class II Analytes. This class of analytes includes phenylthiohydantoin amino acids (PTH-AAs), barbiturates, phenylhydantoin derivatives, coumarinic derivatives, benzoin derivatives, and so forth. In general, class II analytes require moderate micelle polymer concentrations (i.e., defined as 30−50 mM poly-L-SULV) for chiral separations. Table 3 shows that 18 out of 20 racemic class II analytes (∼24% of all analytes investigated) were successfully resolved with R_s values of 1.44−4.92. Figures 2 and 3 show four comparisons of structurally related compounds of class II analytes.

Enantiomeric pairs of eight PTH-AAs were successfully separated as shown in Table 3 with R_s values ranging from 1.46 to 4.92. Although the racemate of each PTH-AA was well separated using 50 mM poly-L-SULV in a 50 mM phosphate/275 mM boric acid (pH 7.2), simultaneous separation of all eight racemates was not achieved in a single run. This is because k' values of some amino acids (e.g., PTH-tyrosine/PTH-norleucine, PTH-phenylalanine) overlapped. Figure 2a shows the simultaneous enanti-

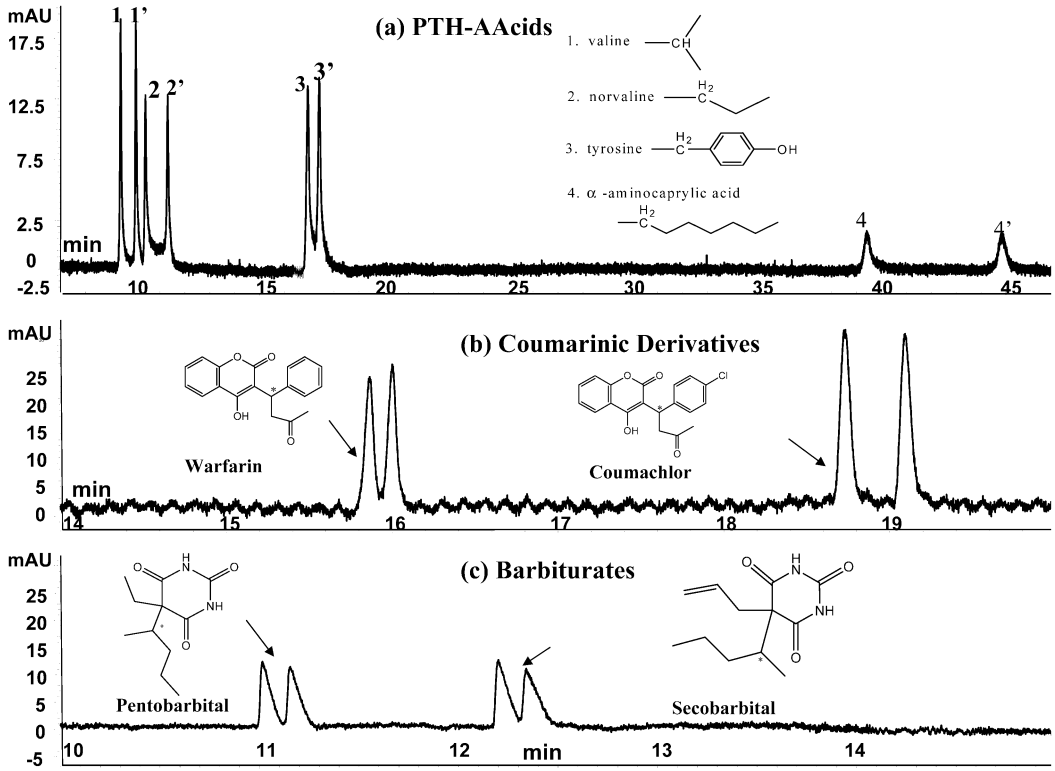


Figure 2. (a) Simultaneous separation and enantioseparation of 0.2 mg/mL each of phenylthiohydantoin amino acids (PTH-AAs). MEKC conditions: 50 mM poly-LV, 15 °C, pH 7.2, 275 mM boric acid, 30 mM Na_2HPO_4, 10 mM triethylamine. Pressure injection, 150 mbar s, +30 kV applied for separation; UV detection at 269 nm. (b) Simultaneous separation and enantioseparation of 0.2 mg/mL each of coumarinic derivatives. MEKC conditions: 50 mM poly-SULV, 15 °C, pH 7.0, 50 mM Na_2HPO_4/NaH_2PO_4. (c) Simultaneous separation and enantioseparation of 0.4 mg/mL each of barbiturates. 50 mM poly-SULV, 15 °C, pH 7.2, 30 mM Na_2HPO_4, 300 mM H_3BO_3. Other conditions in (b) and 2(c) are the same as in(a) except UV detection was performed at 214 nm.

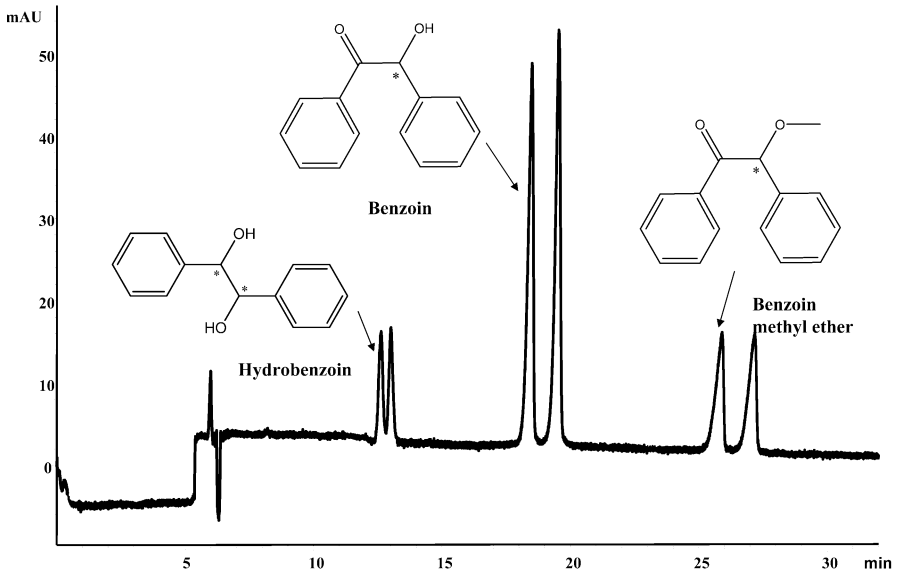


Figure 3. Simultaneous separation and enantioseparation of 0.5 mg/mL each of benzoin derivatives. MCE conditions: same as Figure 2b except UV detection was performed at 220 nm.

oseparation of four PTH AAs. Note that one of the PTH-AAs (PTH-α-aminocaprylic acid) is classified under the class I analytes since it provided high resolution ($R_s = 2.66$) at lower concentrations of poly-L-SULV (i.e., 10 mM, Table 2). However, its R_s value was even higher ($R_s = 9.3$) when separated using the conditions described in Figure 2a. Although the hydrophobicity of the PTH-AAs dictates the elution order as shown in Figure 2a, a similar trend was not observed for the enantiomeric resolution. For example, the most hydrophobic PTH-AAs (PTH-α-aminocaprylic acid) with longest migration time had an exceptionally high R_s value of 9.3. However, under similar conditions, PTH-tyrosine with the third longest migration time provided a R_s value of only 1.4, which is much lower than the R_s value of 4.9 obtained for earlier migrating PTH-norvaline.

In general, anionic chiral compounds are difficult to separate using an anionic chiral selector under neutral or basic conditions. Among the several anionic classes of compounds tested in this study, two classes of compounds (barbiturates and coumarinic derivatives) showed significant enantioresolution with poly-L-SULV. Presumably, favorable chiral interactions between poly-L-SULV and these anionic compounds likely arise from multiple hydrogen-bonding sites present in these two classes of compounds (see Table 3, compounds 17−20). However, electrostatic repulsion cannot be ruled out as a favorable interaction for chiral recognition.

Simultaneous enantioseparation of two coumarinic derivatives (warfarin, coumachlor) is shown in Figure 2b. Warfarin is used as an anticoagulant in the treatment of thromboembolic diseases while coumachlor is used as a rodenticide. Both of these compounds possess multiple hydrogen-bonding moieties and are electronegative due to their keto−enol groups. The phenolic group of warfarin has a pK_a of 5.1.[9] Therefore, these compounds should exist as anions under the conditions (pH 7, 50 mM phosphate buffer) used for the separation. Higher resolution and a longer migration time was achieved with coumachlor relative to warfarin. This is likely attributable to the greater electronegativity of the chlorine group of coumachlor.

Barbiturates (secobarbital, pentobarbital, mephobarbital, hexobarbital) are acidic compounds (pK_a 7.6−8.0) that exist as partially anionic compounds in a phosphate run buffer of pH 7.0. In general, the keto−enol ring systems of barbiturate compounds are chiral if (1) the C-1 carbon possesses two different substituents and (2) one of the keto−enol ring nitrogens is substituted. In the case of pentobarbital and secobarbital, the keto−enol ring system is not chiral because of unsubstitution at the ring nitrogen. Therefore, secobarbital and pentobarbital (compounds 19 and 20, Table 3) possess only one stereogenic center, located on the side chain of the keto−enol ring. The enantioseparation of these compounds resulted in broad peaks under the given conditions (see Figure 2c). Peak broadening may result from the partial dissociation of the barbiturates to the corresponding (anionic) conjugate bases. Another possible reason for the peak broadening is a mobility mismatch between the analytes and the running background electrolyte. In contrast, separations of mephobarbital and hexobarbital (compounds 17 and 18, Table 4) resulted in little to no chiral resolution under all pH conditions (pH 7−10) investigated. Unlike secobarbital and pentobarbital, mephobarbital and hexobarbital possess a stereogenic center within the keto−enol ring system. It appears that the presence of a phenyl group and a cyclohexene group attached to the chiral carbon in mephobarbital and hexobarbital, respectively, causes considerable steric hindrance that inhibits micelle/analyte interactions. Therefore, only slight chiral resolution with much lower k' values (k' = 1.18−1.22) was obtained for mephobarbital and hexobarbital even when the concentration of poly-L-SULV was as high as 85 mM.

The enantiomeric separation of a series of benzoin derivatives was studied to show the effects of steric, hydrophobic, and hydrogen-bonding capabilities of these compounds with poly-L-SULV. Figure 3 shows an electropherogram for the simultaneous separation of (±) hydrobenzoin, (±) benzoin, and (±) benzoin methyl ether. Differences in chiral resolution values of all three benzoin derivatives show that poly-L-SULV exhibits chiral binding interactions that are sensitive to the substituent present at the stereogenic centers. In the case of benzoin and benzoin methyl ether, the presence of the carbonyl group, which acts as a hydrogen-bonding acceptor, decreases the congestion near the stereogenic center. This results in additional rigidity and stronger complexation with poly-L-SULV relative to hydrobenzoin. Further comparison of R_s values indicates that, in the case of hydrobenzoin, the presence of an additional hydroxyl group (hydrogen-bonding acceptor) was not necessary for chiral recognition. This is shown by a relatively lower R_s value obtained for hydrobenzoin (R_s = 1.45) compared to that of benzoin (R_s = 2.95) and benzoin methyl ether (R_s =2.33). It should be noted that the other two derivatives of benzoin (benzoin ethyl ether and benzoin isobutyl ether, Table 3), which are even more hydrophobic, confer unfavorable steric interactions and showed only slight enantioselectivity.

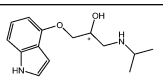
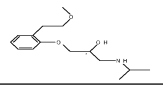
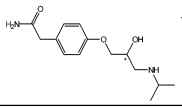
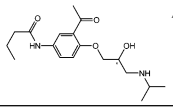
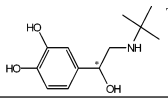
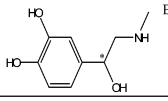
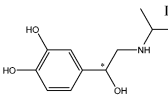
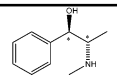
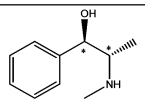
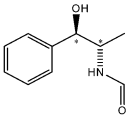
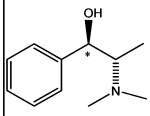
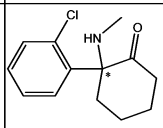
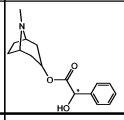
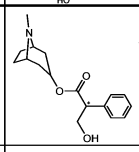
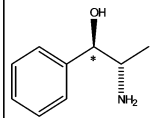
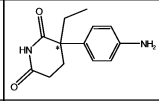
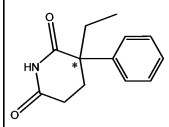
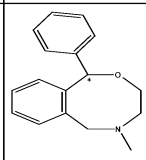
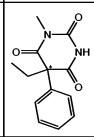
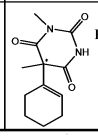
The separation of hydantoin racemates in which the stereogenic center forms a part of the ring system reveals some interesting trends as shown in Table 3. R_s and k' values obtained for the enantiomers of 5-phenylhydantoin derivatives (compounds 9−11, Table 3) showed a marked decrease as the number of aromatic ring systems attached to the stereogenic center drops from two (e.g., 5-(4-hydroxyphenyl)-5-phenylhydantoin, 5-(4-methylphenyl)-5-phenylhydantoin) to one (e.g., 5-methyl-5-phenylhydantoin). Further comparison shows that the presence of a hydroxyl substituent on one of the aromatic rings (e.g., 5-(4-hydroxyphenyl)-5-phenylhydantoin, R_s = 2.23) is more favorable than the presence of a methyl group to the same position of the aromatic ring (e.g., 5-(4-methylphenyl)-5-phenylhydantoin, R_s = 1.86).

Enantioseparation of Class III Analytes. The data for the chiral separations of class III analytes using 75−85 mM poly-L-SULV are tabulated in Table 4. Interestingly, all of the analytes that are enantioseparated in this class (except nefopam) are cationic and exhibit weak chiral interactions with poly-L-SULV. Weak chiral interactions are said to be exhibited by this class since high concentrations of poly-L-SULV (e.g., 75−85 mM) are required to achieve nominal baseline separations with R_s values of 1.33−1.72 and lower migration factors.

All β-adrenergic blockers (β-blockers) contain at least one aromatic ring and an alkanolamine side chain terminating in a secondary amino group which includes the chiral center. Although the three β−blockers (propranolol, alprenolol, oxprenolol) were baseline resolved using only 12.5 mM poly-L-SULV (Table 2), a much higher concentration (i.e., 75 mM) of poly-L-SULV was required for the chiral separations of the other two structural analogues of β-blockers, metoprolol and pindolol (Figure 4a). In addition, it appears that poly-L-SULV exhibits better chiral resolution for β-blockers in which the substituents on the aromatic ring are positioned ortho to the alkanolamine side chain containing the chiral carbon. For example, propranolol, alprenolol, oxprenolol, metoprolol, and pindolol (see Table 3 and Table 4 for structures) all provided baseline resolution either at low or high concentration of poly-L-SULV. In contrast, acebutolol and atenolol in which the substituent on the aromatic ring is positioned para to the alkanolamine side chain (compounds 3 and 4, Table 4) showed only slight chiral resolution.

The chiral resolution, chiral selectivity, and k' values for (±)-epinephrine, (±)-isoproterenol, and (±)-terbutaline are presented in Figure 4b and Table 4. All three secondary amines contain two

Table 4. Class III: Examples of Enantiomers That Exhibit Weak Chiral Interactions with Poly-L-SULV. Enantiomeric Resolution (R_s), Capacity Factors (k'), and Selectivity Factors (α) of Enantiomers Separated with 75−85 mM Poly-L-SULV

Charge of compound under given conditions	Compound	concentration of poly-L-SULV (mM)	Rs	k_2'	α[d]
1. Partially Cationic	 Pindolol[a]	75	1.33	2.84	1.028 (±0.006)
2. Partially Cationic	 Metoprolol[a]	75	1.42	3.23	1.023 (±0.006)
3. Partially Cationic	 Atenolol[a]	75	0.50	2.30	1.010 (±0.002)
4. Partially Cationic	 Acebutolol[a]	75	0.20	2.72	1.010 (±0.001)
5. Partially Cationic	 Terbutaline[a]	75	2.02	3.06	1.052 (±0.008)
6. Partially Cationic	 Epinephrine[a]	75	1.34	1.25	1.022 (±0.003)
7. Partially Cationic	 Isoproternol[a]	75	1.41	1.64	1.033 (±0.006)
8. Partially Cationic	 (±)Ephedrine[a]	75	1.55	2.25	1.019 (±0.00
9. Partially Cationic	 (±)Pseudo-ephedrine[a]	75	1.41	2.35	1.020 (±0.006)
10. Partially Cationic	 (±)Oxyephedrine[a]	75	1.41	2.38	1.020 (±0.006)
11. Partially Cationic	 (±)Methyl-ephedrine[a]	75	1.52	2.75	1.028 (±0.004)
12. Neutral	 Ketamine[a]	75	1.72	1.45	1.029 (±0.003)
13. Cationic	 Homatropine[a]	75	1.52	1.33	1.019 (±0.009)
14. Cationic	 Atropine[a]	75	0.72	1.55	1.025 (±0.006)
15. Partially Cationic	 Norephedrine (Phenylpropanol-amine)[a]	75	1.50	1.92	1.029 (±0.008)
16. Partially Cationic	 Amino-glutethimide[b]	80	5.40	0.88	1.060 (±0.007)
17. Partially Cationic	 Glutethimide[b]	80	1.43	1.42	1.013 (±0.008)
18. Neutral	 Nefopam[b]	80	0.45	1.46	1.011 (±0.006)
19. Partially Anionic	 Mephobarbital[c]	85	0.42	1.18	1.012 (±0.002)
20. Partially Anionic	 Hexobarbital[c]	85	0.22	1.22	1.011 (±0.002)

[a] 50 mM phosphate buffer, pH 6.5, +30 kV, 220 nm, 13 °C, 20 mbar s. [b] 25 mM Tris-borate, pH 8.5, +30 kV, 220 nm, 13 °C, 90 mbar s. [c] 50 mM phosphate buffer, pH 7.2, +30 kV, 220 nm, 90 mbar s. [d] Confidence interval at 90% was calculated for four consecutive injections of each analyte.

hydroxy groups on the aromatic ring adjacent to the chiral carbon. Examination of the electropherogram shows that both the migration time and the enantiomeric resolution of the three amines increase as a function of the bulkiness on the amine functionality in the following order: terbutaline > isoproterenol > epinephrine. The greater resolution obtained for terbutaline relative to the other two chiral amines suggests that the position of the two hydroxyl groups on the aromatic ring may also play a key role in the relative binding of these compounds with poly-L-SULV.

A comparison of chiral resolution observed for glutethimide and its structurally similar analogue, aminoglutethimide (Table 4, Figure 4c), indicates that aminoglutethimide exhibited higher enantioresolution (R_s = 5.40) than glutethimide (R_s = 1.43), despite shorter migration factors (k_2' = 0.88 vs 1.42). This

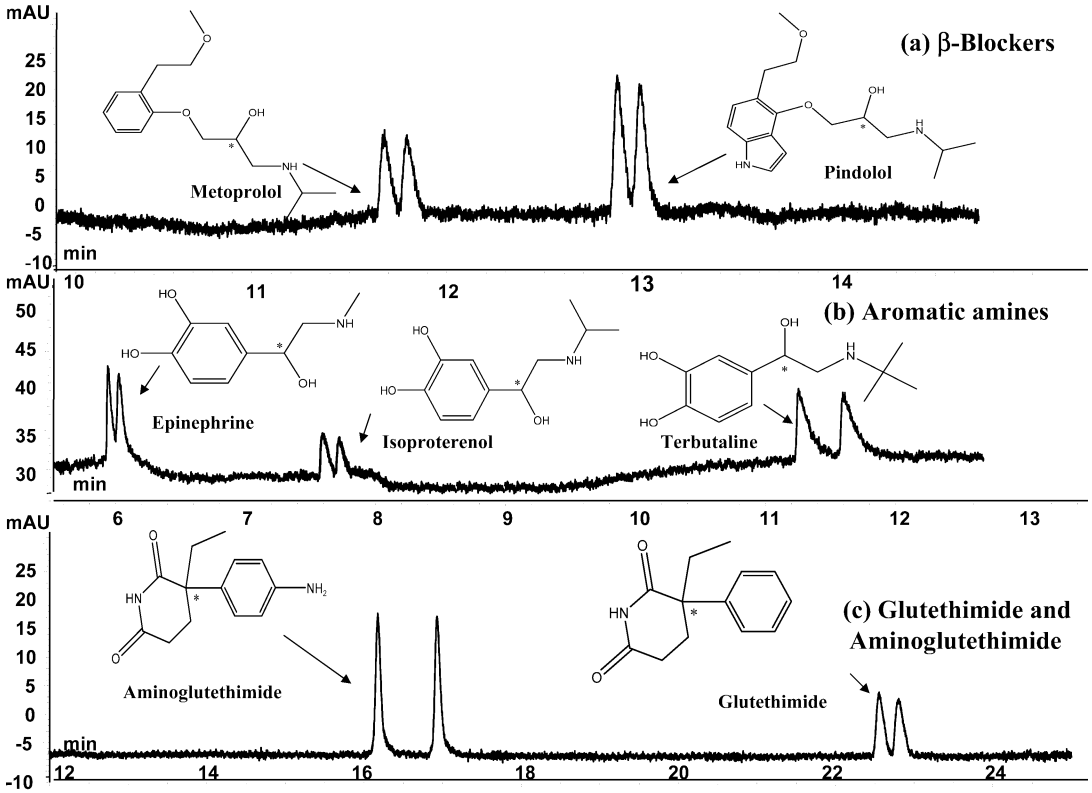


Figure 4. (a) Simultaneous separation and enantioseparation of 0.5 mg/mL each of β-blockers. MCE conditions: 75 mM poly-L-SULV, 14 °C, pH 6.5, 50 mM Na_2HPO_4/NaH_2PO_4. Pressure injection, 30 mbar s, +30 kV applied for separation; UV detection at 220 nm. (b) Simultaneous separation and enantioseparation of 0.5 mg/mL each of aromatic amines. MEKC conditions: same as (a) except UV detection was performed at 214 nm. (c) Simultaneous separation and enantioseparation of 0.5 mg/mL each of aminoglutethimide and glutethimide. MEKC conditions: 80 mM poly-SULV, 12 °C, pH 8.5, 25 mM Tris/Borate. Pressure injection, 90 mbar s, +30 kV applied for separation; UV detection at 254 nm.

comparison of electrophoretic data again points out the importance of electrostatic and hydrogen-bonding interactions of poly-L-SULV that allow for improved chiral resolution.

CONCLUSIONS

According to our current chiral MEKC database, a total of 58 out of 75 racemic compounds of different structural features has been resolved thus far using poly-L-SULV. This represents an overall success rate of ~77%. Among the 58 resolved compounds, 19 (~33%) chiral compounds provided superior chiral resolution ($R_s = 2.05-5.40$), 31 (~54%) chiral compounds provided satisfactory chiral resolution ($R_s = 1.00-1.90$), and only 8 (~13%) chiral compounds provided R_s values less than 1.0. In our laboratory, studies are underway using mixed-micelle polymers of dipeptide surfactants to improve the chiral resolution of compounds that provided either no enantioselectivity or partial enantioselectivity with poly-L-SULV. However, we believe that this superior overall success rate achieved in this chiral screening study will encourage further applications of this highly selective class of dipeptide micelle polymers.

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