Spectroscopic Investigations of Solvent Effect on Chiral Interactions

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The spectrophotometric method was used to determine the mechanism of chiral interactions between a known chiral selector, *tert*-butyl carbamoylated quinine (*t*-BuCQN), and N-derivative amino acids (DNB-Leu). Results obtained on binding constants, free energy of binding (ΔG), and difference in free energy of binding ($\Delta\Delta G$) values seem to suggest that there are three possible types of interactions between DNB-Leu and *t*-BuCQN: electrostatic interaction between the carboxylate group of the DNB-Leu and the ammonium group of the *t*-BuCQN, the donor−acceptor charge-transfer type of interaction between the (acceptor) aromatic group of the amino acid and the (donor) aromatic group of the *t*-BuCQN, and the hydrogen-bonding interaction between the amide group of the DNB-Leu and the carbonyl group of *t*-BuCQN. The strongest interaction will be observed if all of three interactions are in operation as in the case of DNB-Leu. The electrostatic interaction seems to play the dominant role in the interactions. While the charge-transfer interaction is relatively weaker, it seems, however, to be responsible for enantiomeric selectivity, namely, the closer the electron acceptor dinitrophenyl group is to the electron donor quinoline group, the higher is the enantiomeric selectivity. Specifically, in solvent with high polarity, both donor and acceptor are solvated by solvent molecules, thereby preventing them from being close. As a consequence, the interaction will be weaker and, hence, lower enantiomeric selectivity. Solvation will be less in less polar solvent which, in turn, leads to stronger interaction and higher enantiomeric selectivity.

Introduction

Chiral recognition is important in many different fields including chemistry, biology, pharmaceutical, and medical sciences.[1−6] It has long been recognized that specificity and efficacy of many biologically important reactions are based on chiral interactions.[1−6] Recognizing the importance of chiral effects, the FAA in 1992 issued a mandate requiring pharmaceutical companies to evaluate effects of individual enantiomers of chiral drugs that are produced. It is thus hardly surprising that the pharmaceutical industry needs effective methods to evaluate chiral interactions.

Chiral discrimination can be investigated by a variety of spectroscopic methods including spectrophotometry, fluorescence, circular dichroism, and NMR.[7−11] The spectrophotometric method is widely used as it is nonintrusive (i.e., no need to add any label reagents such as those often used in fluorescence and NMR methods) and is applicable to many compounds. In fact, recently we have successfully used the spectrophotometric method not only to determine binding constants but also to eluciadate mechanism of enantiomeric interactions between cyclodextrins and many different substrates in H_2O as well as in room-temperature ionic liquids.[12−14] Results from our earlier studies clearly demonstrate that the spectrophotometric method can be used for the determination of mechanism of chiral interactions for other systems as well. Such consideration prompted us to initiate this study, which aims to use the method to determine mechanism of chiral interactions in other important systems such as those responsible for chiral separations in liquid chromatography and capillary electrophoresis.

Carbamoylated derivatives of quinine are effective as chiral selectors for the enantiomeric separation of N-derivative amino acids by capillary electrophoresis (CE) as well as by HPLC.[15−18] As a consequence, various studies have been performed to determine the mechanism of enantiomeric selectivity of this promising chiral selector to optimize its utilization and expand its applications. Unfortunately, to date, results of these studies fail to provide unequivocal information on the mechanism of the chiral interactions. This type of information is of particular importance as it will provide knowledge needed not only to guide and to optimize but also to expand applications of this particular chiral selector and other chiral selectors for HPLC and CE.

The aim of our study is to use the spectroscopic technique to systematically investigate interactions between the quinine chiral selector and amino acids, to determine their binding constants, and to utilize solvent effect to gain insight into interactions. Preliminary results are reported in this manuscript.

Experimental Section

Chemicals. Methanol (HPLC grade) and acetonitrile were obtained from Alfa Aesar and were distilled before use. 1-Butanol (Aldrich) was distilled before use. Absolute ethanol was dried with CaH_2 and was distilled before used. All the other solvents were also distilled before use. Quinine was purchased from Aldrich. *tert*-Butyl carbamoylated quinine (*t*-BuCQN) was synthesized according to a previously published precedure.[19] Methyl esters of (*R*)-leucine and (*S*)-leucine were purchased from NOVA Biochem. (*S*)-DNB-Leu was purchased from Aldrich and the corresponding R enantiomer was synthesized from the D-Leu (purchased from Avocado) and 3,5-dinitrobenzoyl chloride (purchased from Aldrich) according to a procedure described previously.[20] Methyl esters of (*S*)-DNB-Leu (and (*R*)-DNB-Leu) were synthesized using reported procedure.[21] Essentially, DNB-leucine was dissolved in a large excess of

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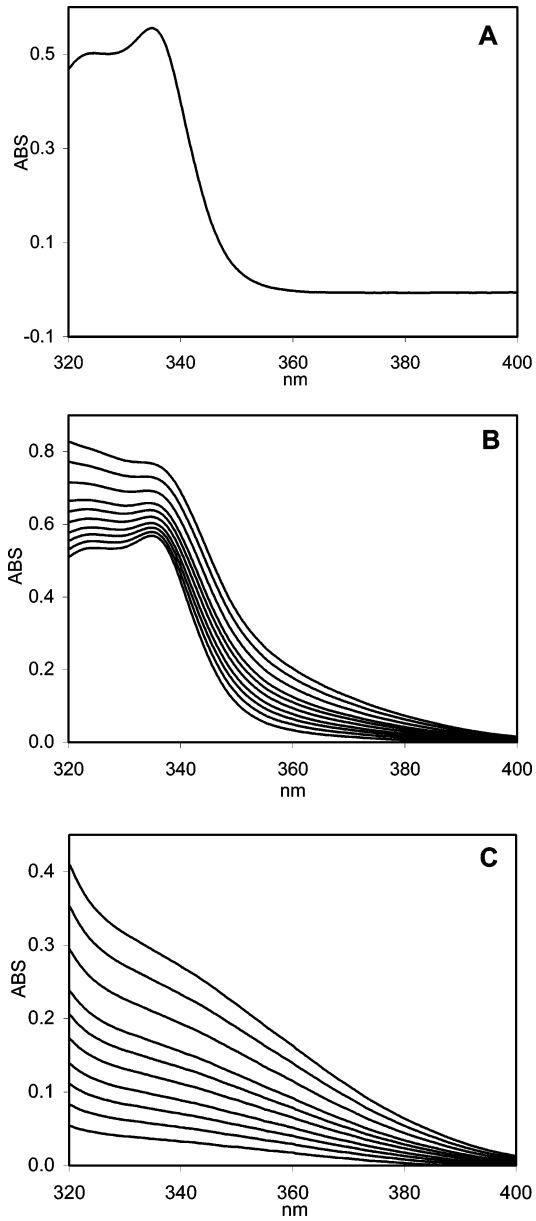

Figure 1. Absorption spectra of 1-butanol solution of (A) 0.10 mM of *t*-BuCQN, (B) mixtures of 0.10 mM of *t*-BuCQN with different concentrations of DNB-Leu (from 0.20 to 2.00 mM), and (C) different concentrations of DNB-Leu (from 0.20 to 2.00 mM) without *t*-BuCQN.

absolute methanol that was saturated with HCl gas (HCl saturated methanol solution was prepared from dried methanol, NaCl, and concentrated H_2SO_4). The solution was refluxed for 1.0 h and the solvent was removed under vacuum and the residue was recrystallized from acetonitrile. The yield was about 70%. $[\alpha]_D^{20} = -19.6 \pm 0.3$ and $+20.7 \pm 0.3$ for *S*- and *R*-DNB-Leu-OMe, respectively ($c = 0.31828$, MeOH). For methyl ester of (*S*)-DNB-Leu, ^{1}HNMR ($CDCl_3$): 9.172 (t, 1H), 8.933 (d, 2H), 6.854 (d, 1H), 4.872 (m, 1H), 3.808 (s, 3H), 1.744 (m, 2H), 0.994 (dd, 6H). For methyl ester of (*R*)-DNB-Leu, ^{1}HNMR ($CDCl_3$): 9.163 (t, 1H), 8.912 (d, 2H), 6.995 (d, 1H), 4.869 (m, 1H), 3.813 (s, 3H), 1.743 (m, 2H), 0.988 (dd, 6H). *R*- and *S*-benzoyl-Leu-OMe were synthesized from benzoyl chloride with *R*- and *S*-Leu-OMe. Similarly, *R*-acetyl-Leu-OMe and *S*-acetyl-Leu-OMe were synthesized from acetyl chloride and *R*- and *S*-Leu-OMe.

Absorption spectra were taken at room temperature in a 1-cm path length cell using a Shimadzu UV-2501 absorption spectrometer.

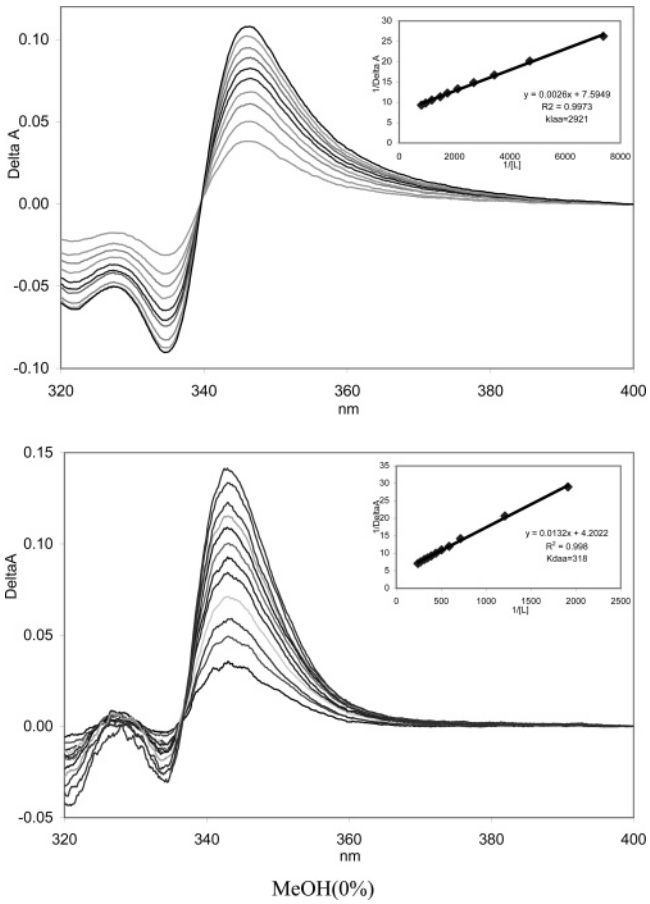

Figure 2. Differential spectra of *t*-BuCQN with either *S*-DNB-Leu (top) or *R*-DNB-Leu (bottom) in 1-butanol. Inserts are corresponding Benesi−Hilderbrandt double reciprocal plots. See text for detailed information.

Results and Discussion

Absorption spectrum of 0.10 mM solution of *t*-BuCQN in 100% 1-butanol is shown in Figure 1A. As illustrated, this quinine derivative exhibits a strong absorption with a maximum at about 338 nm. It was found that the absorption spectrum of *t*-BuCQN underwent changes when DNB-Leu was added into the solution. This indicates that *t*-BuCQN forms complexes with DNB-Leu and that this complex formation produces observable changes in the absorption spectra. As described in our earlier studies,[12−14] such absorption changes can be used to determine binding constants. In this case, the binding constants were determined by initially recording absorption spectra in the 320−400 nm region of a set of solutions containing a constant concentration of *t*-BuCQN (e.g., 0.10 mM) and different concentrations of (*R*)-DNB-Leu (or (*S*)-DNS-Leu) (or other enantiomers of leucine derivatives) with concentrations ranging from 0.20 to 2.00 mM (set 1, Figure 1B). Spectra of a second set (set 2, Figure 1C) of solutions containing the same (*R*)-DNB-Leu concentrations but without *t*-BuCQN were also taken. There is a corresponding pair from these two sets, each pair having the same concentration of (*R*)-DNB-Leu, one with *t*-BuCQN (set 1) and the other without (set 2). Differential absorption spectrum (ΔA) was obtained for each pair by subtracting spectrum of set 2 (Figure 1C) and *t*-BuCQN spectrum (Figure 1A) from that of the corresponding spectrum in set 1 (Figure 1B). The resulting set of differential spectra shown in Figure 2A reflect the absorbance change (ΔA) as a function of added (*R*)-DNB-Leu. For comparison, another set of differential spectra was also measured under identical

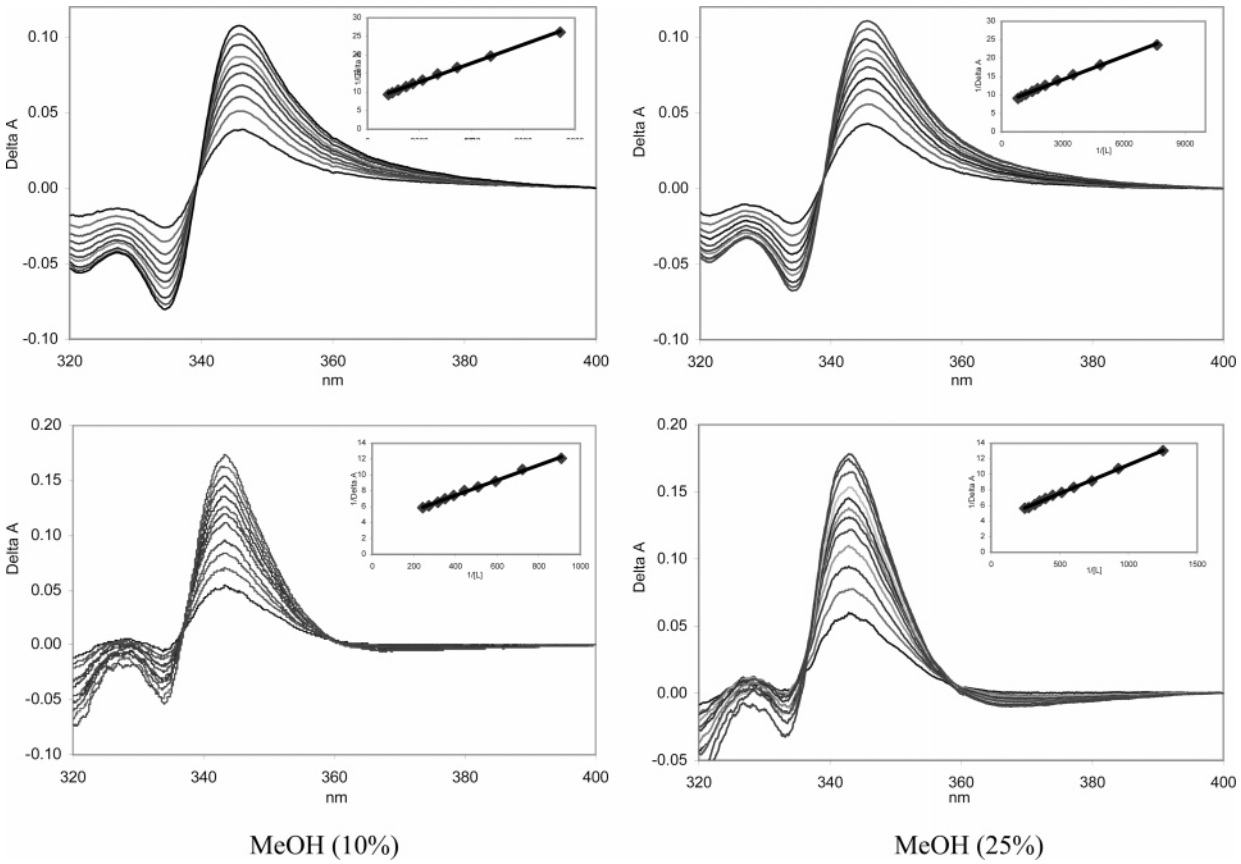

Figure 3. Differential spectra of *t*-BuCQN with either *S*-DNB-Leu (top) or *R*-DNB-Leu (bottom) in 10:90 methanol:butanol (left) and 25:75 methanol:butanol (right). Inserts are corresponding Benesi−Hilderbrandt double reciprocal plots. See text for detailed information.

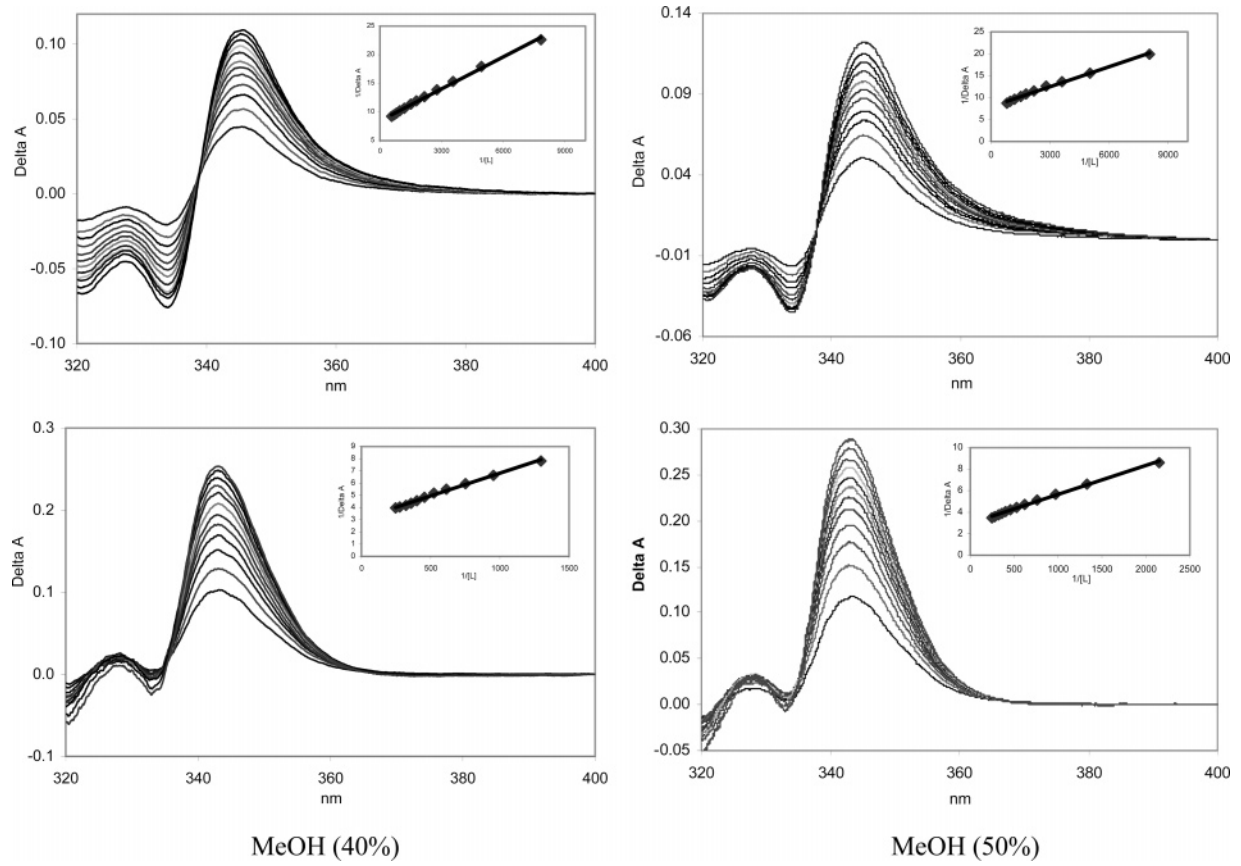

Figure 4. Differential spectra of *t*-BuCQN with either *S*-DNB-Leu (top) or *R*-DNB-Leu (bottom) in 40:60 methanol:butanol (left) and 50:50 methanol:butanol (right). Inserts are corresponding Benesi−Hilderbrandt double reciprocal plots. See text for detailed information.

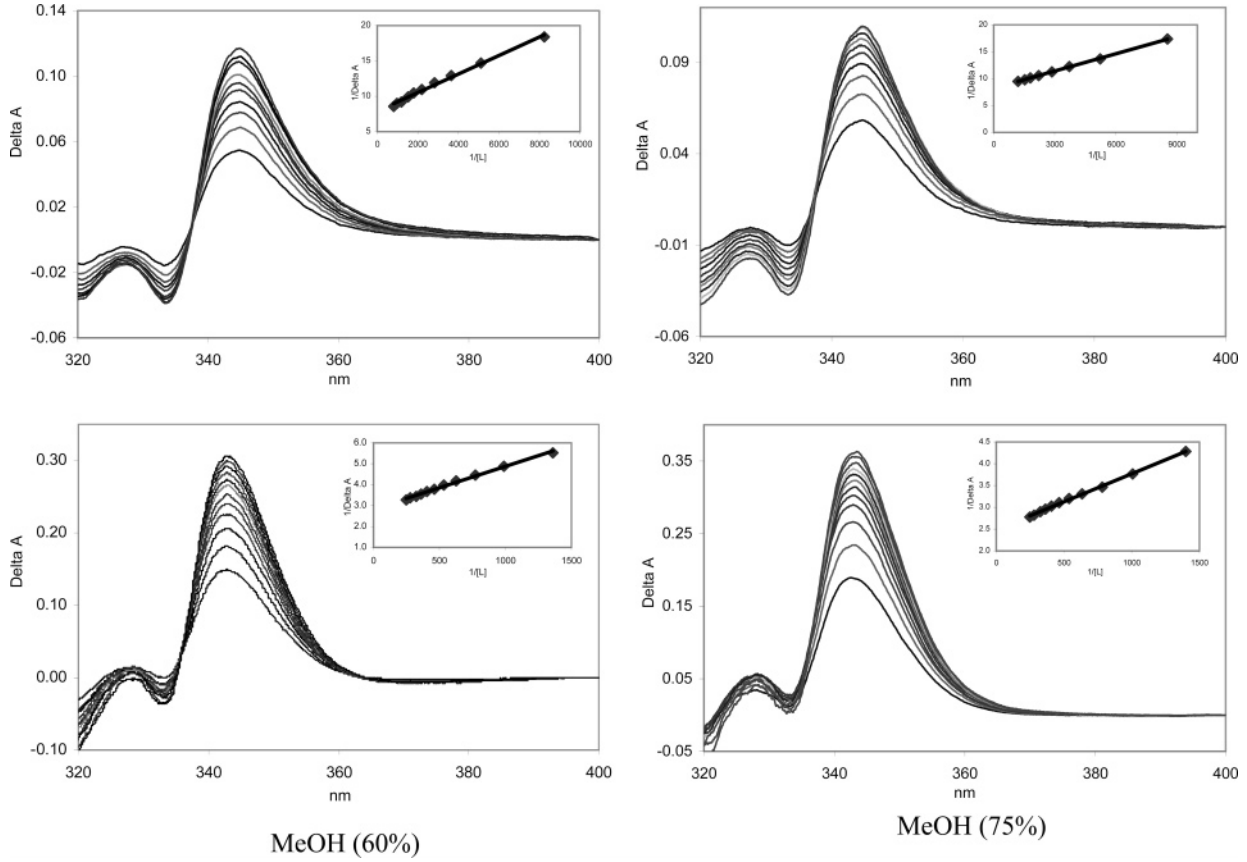

Figure 5. Differential spectra of *t*-BuCQN with either *S*-DNB-Leu (top) or *R*-DNB-Leu (bottom) in 60:40 methanol:butanol (left) and 75:25 methanol:butanol (right). Inserts are corresponding Benesi−Hilderbrandt double reciprocal plots. See text for detailed information.

concentrations and experimental conditions but for another enantiomer, namely, between (*S*)-DNB-Leu and *t*-BuCQN (Figure 2B). There are significant differences between these two sets of spectra. Not only do maximum absorbance changes occur at different wavelengths (342 nm for (*R*)-DNB-Leu and 346 nm for (*S*)-DNB-Leu), but also the magnitudes of absorbance changes are different as well. It is, therefore, evidently clear that interactions of *t*-BuCQN with (*R*)-DNB-Leu are different from those with (*S*)-DNB-Leu. In fact, such a large difference in interactions should lead to a large difference in binding constants.

Similar to the method used in our previous publications,[12−14] binding constants (*K*) were calculated by linearization of changes in ΔA versus [*L*], where [*L*] is the concentration of either *R*-DNB-Leu or *S*-DNB-Leu, using the Benesi−Hilderbrandt double reciprocal plot.

$$\frac{1}{\Delta A} = \frac{1}{S\Delta\epsilon K[L]} + \frac{1}{S\Delta\epsilon}$$

S is the concentration of *t*-BuCQN, and $\Delta\epsilon$ is the change in molar absorptivity upon complex formation.[12−14] Shown as inserts in Figure 2A and 2B are the Benisi−Hilderbrandt plots for *S*-DNB-Leu and *R*-DNB-Leu, respectively. From these plots, binding constants between *t*-BuCQN and *R*-DNB-Leu and *S*-DNB-Leu were determined to be 2921 and 318 M^{-1}, respectively. The results clearly indicate that the interactions between *t*-BuCQN and DNB-Leu are strongly dependent on the stereochemistry of the amino acid. Chiral recognition can produce up to a 9-fold difference in binding constants!

Effect of solvent polarity[22,23] on binding constants of *t*-BuCQN with both enantimeric forms of DNB-Leu was then investigated to gain insight into chiral recognition. This was accomplished by measuring binding constants in mixtures of methanol and 1-butanol with different ratios. Shown in Figures 3−6 are differential absorption spectra for (top) *t*-BuCQN and *S*-DNB-Leu and (bottom) *t*-BuCQN and *R*-DNB-Leu in 10:90, 25:75, 40:60, 50:50, 60:40, 75:25. 90:10, and 0:100 methanol:butanol mixtures. Significant enantiomeric differences in the spectra are observed in all mixtures. However, the differences, in terms of maximum wavelength, are relatively smaller than that in pure 1-butanol. For example, the λ_{max} value for *t*-BuCQN and *S*-DNB-Leu was shifted from 346 nm (in pure butanol) to 345 nm in 10:90 methanol:butanol mixture and continued shifting down to 343 nm in pure methanol. Of particular interest is the fact that the λ_{max} values for *t*-BuCQN and *R*-DNB-Leu remain constant at 342 nm in all methanol:butanol mixtures. It seems that the difference in the λ_{max} values between both enantiomers decreases as the relative concentration of methanol increases or as the solvent polarity increases.[22,23] Binding

TABLE 1: Binding Constants (*K*), Binding Energies (ΔG), and Binding Energy Differences ($\Delta\Delta G$) for Complexes of *t*-BuCQN with either (*S*)-DNB-Leu or (*R*)-DNB-Leu in Methanol and 1-Butanol Mixtures

MeOH (%)	pol-arity[22,23]	$K_{(S)-DNB-Leu}$ (M^{-1})	$K_{(R)-DNB-Leu}$ (M^{-1})	$-\Delta G_S$ kJ/mol	$-\Delta G_R$ kJ/mol	$\Delta\Delta G$ (kJ/mol)
100	6.60	9421 ± 236	3431 ± 105	22.82	20.30	2.52
90	6.33	8614 ± 49	2695 ± 71	22.60	19.70	2.90
75	5.93	7441 ± 37	1911 ± 31	22.24	18.85	3.39
60	5.52	6073 ± 213	1415 ± 39	21.73	18.10	3.63
50	5.25	5302 ± 182	1095 ± 29	21.39	17.46	3.93
40	4.98	4460 ± 102	834 ± 24	20.96	16.78	4.18
25	4.58	3711 ± 114	533 ± 12	20.50	15.66	4.84
10	4.17	3071 ± 67	382 ± 13	20.03	14.83	5.20
0	3.90	2921 ± 61	318 ± 13	19.90	14.37	5.53

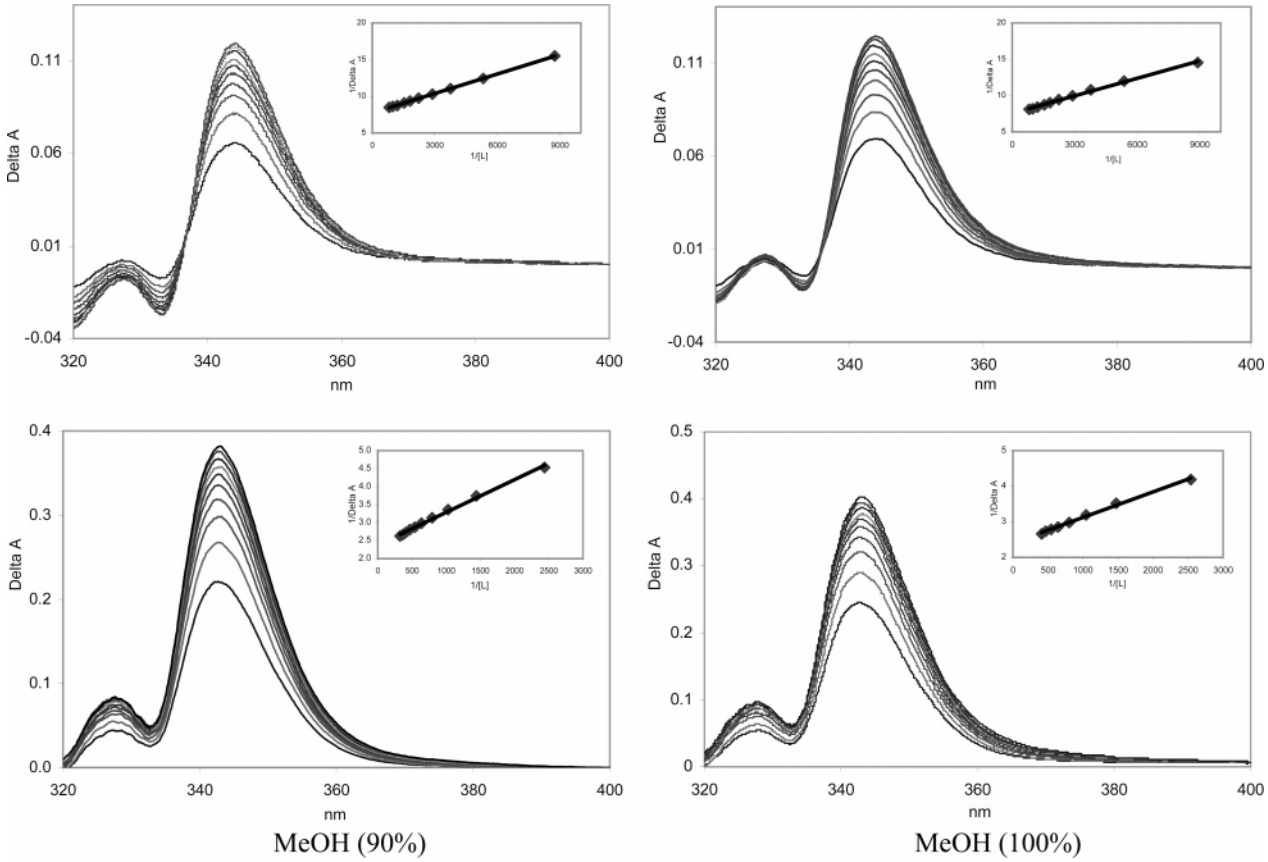

Figure 6. Differential spectra of *t*-BuCQN with either *S*-DNB-Leu (top) or *R*-DNB-Leu (bottom) in 90:10 methanol:butanol (left) and 100% methanol (right). Inserts are corresponding Benesi−Hilderbrandt double reciprocal plots. See text for detailed information.

constants were calculated from double reciprocal plots shown as inserts in the figures, and the results are listed in Table 1. It is evident from the table (and also from Figure 7A which plots binding constants as a function of solvent polarity[22,23] or methanol content of methanol:butanol mixtures) that increasing polarity of the solvent (i.e., increasing relative methanol content) leads to an increase in the binding constant. However, as evident from Figure 7A, for both enantiomers, binding constants are not linearly related to solvent polarity. For example, for *S*-DNB-Leu, the *K* value increases by 1.81-fold and 3.2-fold when methanol content was increased from 0% to 50 and 100% (*K* changes from 2921 to 5302 and 9421, respectively) whereas for *R*-DNB-Leu, it increases from 318 to 1095 (3.4-fold) and to 3431 (10.8-fold), respectively. While an increase in the solvent polarity[22,23] leads to an increase in the binding constant, it also produces an undesirable effect, namely, it decreases enantiomeric recognition in *K* values. Specifically, the difference between *K* values of *S*-DNB-Leu and *R*-DNB-Leu was 9.1-fold in 0% methanol but decreases to 4.8-fold and to 2.7-fold in 50% and 100% methanol mixtures, respectively.

Table 1 also lists values of free energy of binding (ΔG) for both *R*- and *S*-DNB-Leu. ΔG values were calculated from binding constants *K* by $\Delta G = -RT \ln(K)$. The change in the $-\Delta G$ value is similar to that of the binding constants, namely, they both increase as solvent polarity[22,23] increases. However, correlations of these two values with solvent polarity are different. Specifically, $-\Delta G$ values for both *R*- and *S*-DNB-Leu exhibit linear relationship with solvent polarity whereas *K* varies exponentially (Figure 7A and B). This type of linear relationship is particularly useful because it may be possible to use this relationship to calculate not only the free energy of binding but also the binding constant between either *R*- or *S*-DNB-Leu with *t*-BuCQN in other solvent mixtures with known polarity.

It is evident that the type of binding (strong or weak) can be predicted from the free energy of binding ($-\Delta G$). However, it does not provide information on the enantiomer selectivity in the binding. Such information can be deduced from the difference in free energy of binding ($\Delta\Delta G$) between *S*- and *R*-DNB-Leu. $\Delta\Delta G$ was calculated from $\Delta\Delta G = \Delta G_S - \Delta G_R$, where ΔG_S and ΔG_R are free energy of binding for *S*- and *R*-DNB-Leu with *t*-BuCQN, respectively. Calculated $\Delta\Delta G$ values are listed in Table 1 whereas Figure 7C shows a plot of $\Delta\Delta G$ as a function of solvent polarity.[22,23] Similar to ΔG, $\Delta\Delta G$ is linearly related to solvent polarity. However, the linear correlation between $\Delta\Delta G$ with solvent polarity is in the opposite direction with that of ΔG. Specifically, while binding between *t*-BuCQN with either *R*- or *S*-DNB-Leu is stronger in more polar solvent, enantiomer selectivity is higher in solvent with low polarity.

To verify if this type of correlation is general and is applicable to other solvent mixtures as well or is only specific to mixtures of methanol:butanol, binding constants for *t*-BuCQN with *R*-DNB-Leu and with *S*-DNB-Leu in methanol:ethanol mixtures with various ratios were also determined. Calculated binding constants, ΔG and $\Delta\Delta G$ values, for methanol:ethanol mixtures at various ratios from 0% to 100% are listed in Table 2. It is interesting to compare these results with those for methanol:butanol mixtures. It is pleasing to observe that the *K* values for *S*- and *R*-DNB-Leu in 100% methanol determined here ((9800 ± 239) M^{-1} and (3431 ± 105) M^{-1}, respectively) agree very well, within experimental error, to the values of (9421 ± 236) M^{-1} and (3431 ± 105) M^{-1}, previously determined for the mixture of methanol:butanol (Table 1). This agreement lends

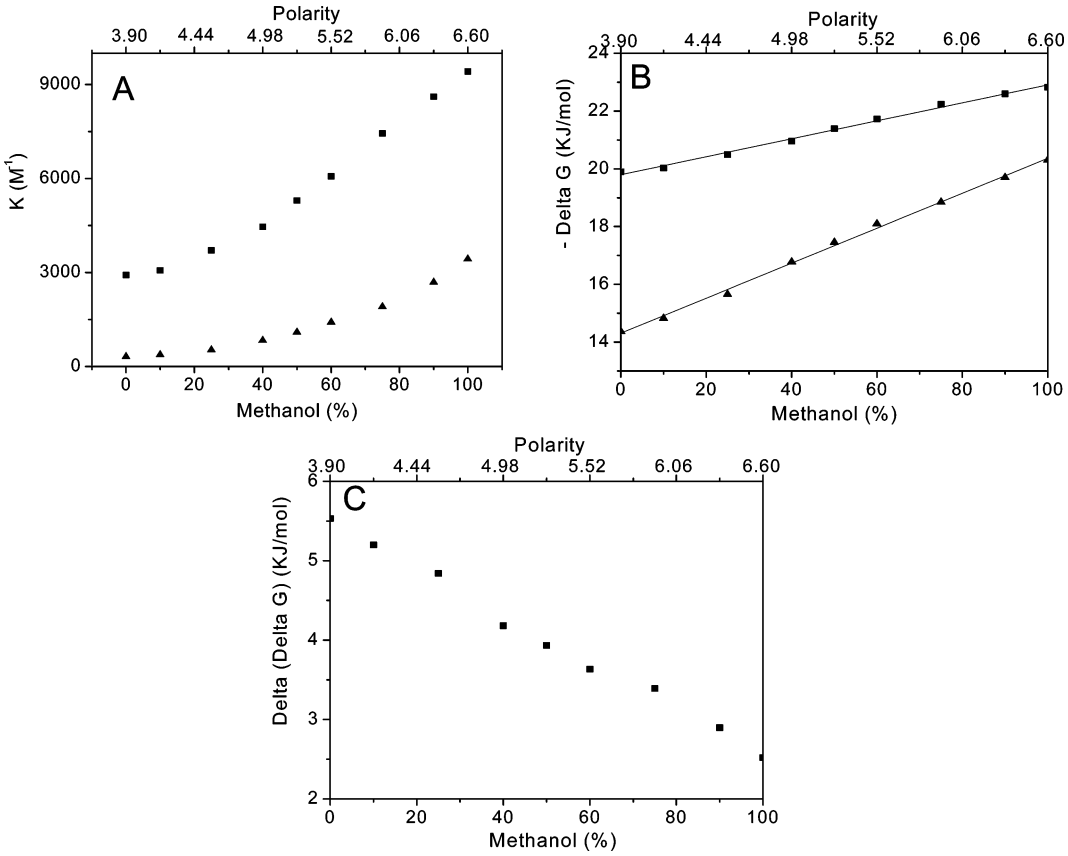

Figure 7. Plots of binding constant (K), free energy of binding (ΔG), and binding energy difference ($\Delta\Delta G$) as a function of methanol content (of methanol:butanol mixtures) and solvent polarity for complexes of *t*-BuCQN with either *R*-DNB-Leu (▲) or *S*-DNB-Leu (■).

TABLE 2: Binding Constants (K), Binding Energies (ΔG), and Binding Energy Differences ($\Delta\Delta G$) for Complexes of *t*-BuCQN with either (*S*)-DNB-Leu or (*R*)-DNB-Leu in Methanol and Ethanol Mixtures

methanol (%)	polarity[22,23]	$K_{(S)-DNB-Leu}$ (M^{-1})	$K_{(R)-DNB-Leu}$ (M^{-1})	$-\Delta G_S$ kJ/mol	$-\Delta G_R$ kJ/mol	$\Delta\Delta G$ (kJ/mol)
0	5.20	2553 ± 42	570 ± 13	19.57	15.83	3.74
25	5.55	3504 ± 44	865 ± 20	20.36	16.87	3.49
40	5.76	4331 ± 107	1144 ± 24	20.89	17.57	3.32
50	5.90	5095 ± 74	1362 ± 42	21.29	18.00	3.29
75	6.25	7208 ± 152	2132 ± 45	22.16	19.12	3.04
90	6.46	8196 ± 117	2773 ± 27	22.48	19.77	2.70
100	6.60	9800 ± 239	3431 ± 105	22.92	20.30	2.62

credence to the accuracy of our method. Additional information on the binding mechanism can be obtained by comparing results here with those in methanol:butanol mixtures. Take, for example, results in the 50:50 mixture of methanol:ethanol (Figure 8). This mixture has a polarity[22,23] of 5.90 which is relatively similar to that of a 75:25 mixture of methanol:butanol. While these two mixtures have similar polarity and differential absorption spectra (not shown), the binding constants are distinctly different, namely, K values for *S*-DNB-Leu and *R*-DNB-Leu in methanol:ethanol mixture were 5095 and 1362 M^{-1}, respectively, which are somewhat smaller than values of 7441 and 1911 M^{-1} in methanol:butanol mixture. In fact, when K and ΔG values in methanol:ethanol mixtures were plotted against solvent polarity together with those for methanol:butanol (Figure 9 A and B), it was found that at the same solvent, K as well as ΔG values in methanol:ethanol mixture are always lower than those in methanol:butanol mixtures. Of particular interest is the results shown in Figure 9C which plots $\Delta\Delta G$ as a function of solvent polarity for both mixtures. As illustrated, not only is $\Delta\Delta G$ linearly related to solvent polarity for methanol:ethanol mixtures, but it also has the same linear relationship as that for methanol:butanol mixtures. The results seem to indicate that within the solvent systems used in this study, binding of *R*- and *S*-DNB-Leu with *t*-BuCQN is dependent on the polarity as well as on the structure of solvents. Enantiomeric selectivity, however, depends only on the solvent polarity.

The role of the carboxylic acid group in the binding interactions was then investigated by replacing *R*- and *S*-DNB-Leu with their corresponding methyl esters (*R*- and *S*-DNB-Leu-OMe). Binding constants of the esters with *t*-BuCQN were then measured in various solvents. Surprisingly, it was found that *R*- and *S*-DNB-Leu-OMe do not have much interaction with *t*-BuCQN. In fact, in some solvents including mixtures of methanol:butanol and methanol:ethanol, the interactions are so weak that it was not possible to measure binding constants accurately. Measurable binding constants for some solvents are listed in Table 3. It is evident from the table that bindings of the esters are 2 orders of magnitudes lower than those for the DNB-Leu. For example, in methanol, *S*-DNB-Leu binds to *t*-BuCQN with a K value of 9421 M^{-1} whereas the binding constant of its methyl ester is only 10 M^{-1}. However, although *t*-BuCQN can only interact very weakly with DNB-Leu-OMe (i.e., low binding constants), it does offer some enantimeric selectivity. For example, in 50:50 chlorobutane:cyclohexane, K values for *S*-DNB-Leu-OMe are 29 M^1 which is more than 7 times higher than that for *R*-DNB-Leu-OMe.

To elucidate the effect of the nitro groups on the aromatic ring of the DNB-Leu-OMe, binding between *R*- and *S*-benzoyl-Leu-OMe with *t*-BuCQN was then investigated. It appears that *R*- and *S*-benzoyl-Leu-OMe do not bind to *t*-BuCQN at all because no measurable changes were observed on the spectrum of *t*-BuCQN in all solvents when the amino acid derivatives were added into it. The fact that DNB-Leu-OMe binds to

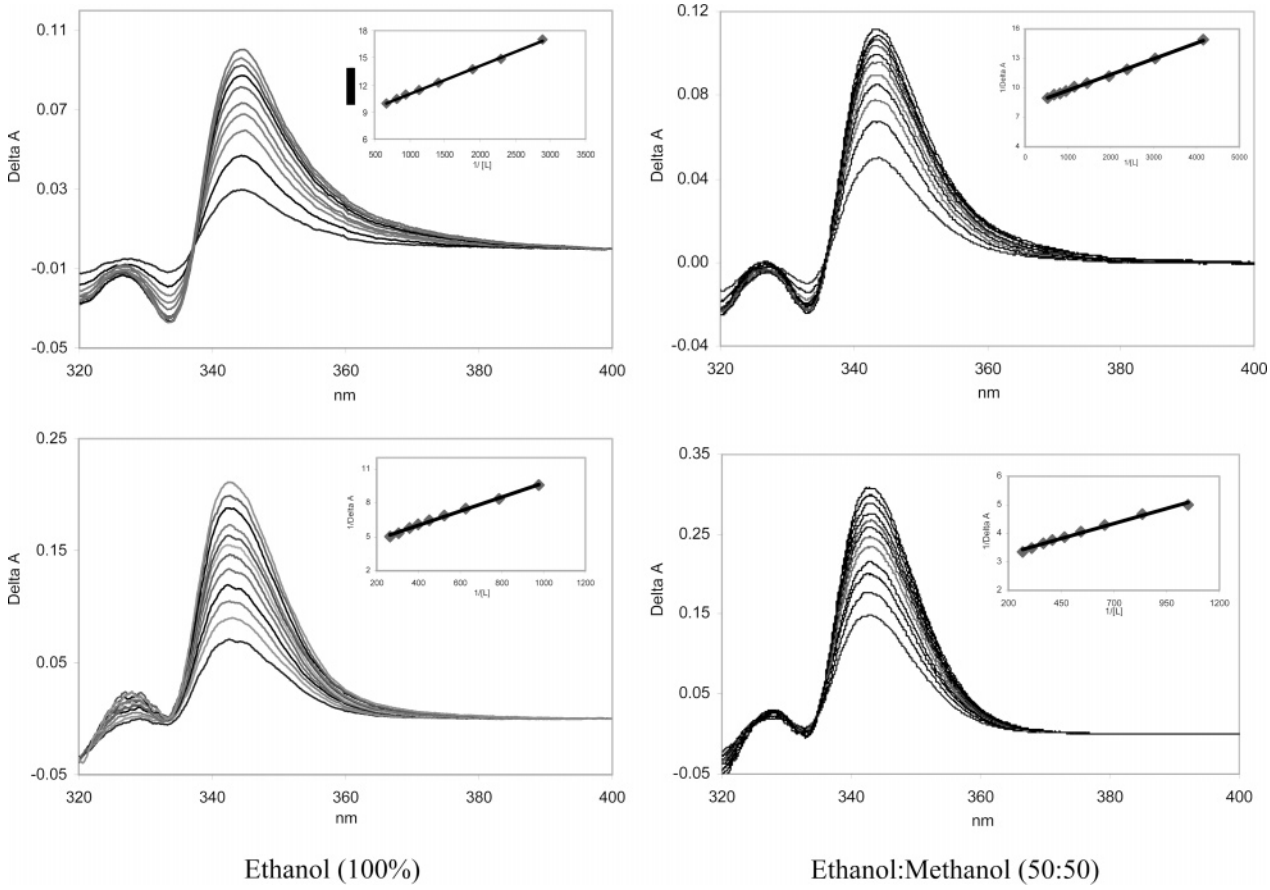

Figure 8. Differential spectra of *t*-BuCQN with either *S*-DNB-Leu (top) or *R*-DNB-Leu (bottom) in 100% ethanol (left) and 50:50 methanol: ethanol mixture (right). Inserts are corresponding Benesi−Hilderbrandt double reciprocal plots. See text for detailed information.

t-BuCQN but not benzoyl-Leu-OMe seems to suggest that the DNB group is involved in the binding interactions and that the binding is induced by the electron-withdrawing effect of its nitro groups.

Binding between *R*- and *S*-acetyl-Leu-OMe with *t*-BuCQN was then investigated to gain insight into the role of the amide group in the binding interactions. It was found that acetyl-Leu-OMe does not have much interaction with *t*-BuCQN. The interactions are so weak in some solvents such as mixtures of methanol:butanol and methanol:ethanol that there were no observable changes in the spectra of *t*-BuCQN when the amino acids were added. Only in solvents with relatively higher polarity such as methanol and acetonitrile were there some small spectral changes to facilitate calculation of binding constants. *K* values for *R*- and *S*-acetyl-Leu-OMe with *t*-BuCQN were, respectively, (1.5 ± 0.5) M^{-1} and (1.2 ± 0.6) M^{-1} in methanol and (1.6 ± 1.1) M^{-1} and (1.1 ± 0.2) M^{-1} in acetonitrile. *R*- and *S*-benzoyl-Leu-OMe do not bind to *t*-BuCQN in all solvents used in this study. The fact that Ace-Leu-OMe can bind but benzolyl-DNB-Leu-OMe cannot bind to *t*-BuCQN even though both compounds can, in principle, form hydrogen bonds between their amide groups and the carbonyl group of *t*-BuCQN seems to suggest that benzoyl-Leu-OMe cannot form any hydrogen bond because of the steric hinder by its bulky aromatic group. While DNB-Leu-OMe is structurally similar to benzoyl-Leu-OMe, it can, however, bind weakly to *t*-BuCQN. This is, as explained in the previous paragraph, may be because DNB-Leu-OMe has the electron-withdrawing nitro groups on its aromatic ring. This makes it possible for DNB-Leu-OMe to bind to *t*-BuCQN through change-transfer interaction between its electron acceptor aromatic ring and the electron donor aromatic ring of the *t*-BuCQN.

Conclusions

It has been demonstrated that the spectrophotometric method can be successfully used to determine the mechanism of chiral interactions between *tert*-butyl carbamoylated quinine (*t*-BuCQN) and amino acid derivatives. On the basis of determined binding constants, free energy of binding (ΔG), and difference in free energy of binding ($\Delta\Delta G$) values between *t*-BuCQN and amino acid in various solvent mixtures with different polarity, it seems that within the solvent systems used in this study, the binding is stronger in a solvent with higher polarity but the enantiomeric selectivity is higher in a solvent with lower polarity. Among amino acid derivatives, DNB-Leu offers the strongest binding and highest enantiomeric selectivity. When the carboxylate group is replaced by an ester group as in DNB-Leu-OMe, the binding with *t*-BuCQN is relatively weaker, but there are some degrees of enantiomeric selectivity. Replacing the DNB group by either an acetyl group or a benzoyl group leads to either a substantial decrease in the binding (acetyl-Leu-OMe) or no binding at all (benzoyl-Leu-OMe). Taken together, these results seem to suggest that there are three possible types of interactions between DNB-Leu and *t*-BuCQN: electrostatic interaction between the carboxylate group of the DNB-Leu and the ammonium group of the *t*-BuCQN, the donor−acceptor charge-transfer type of interaction between the (acceptor) aromatic group of the amino acid and the (donor) aromatic group of the *t*-BuCQN, and the hydrogen-bonding interaction between the amide group of the DNB-Leu and the carbonyl group of *t*-BuCQN (see Figure 10). The fact that DNB-Leu has much higher binding constants than DNB-Leu-OMe implies that the electrostatic interaction is the dominant type of interaction. While the charge-transfer interaction is relatively

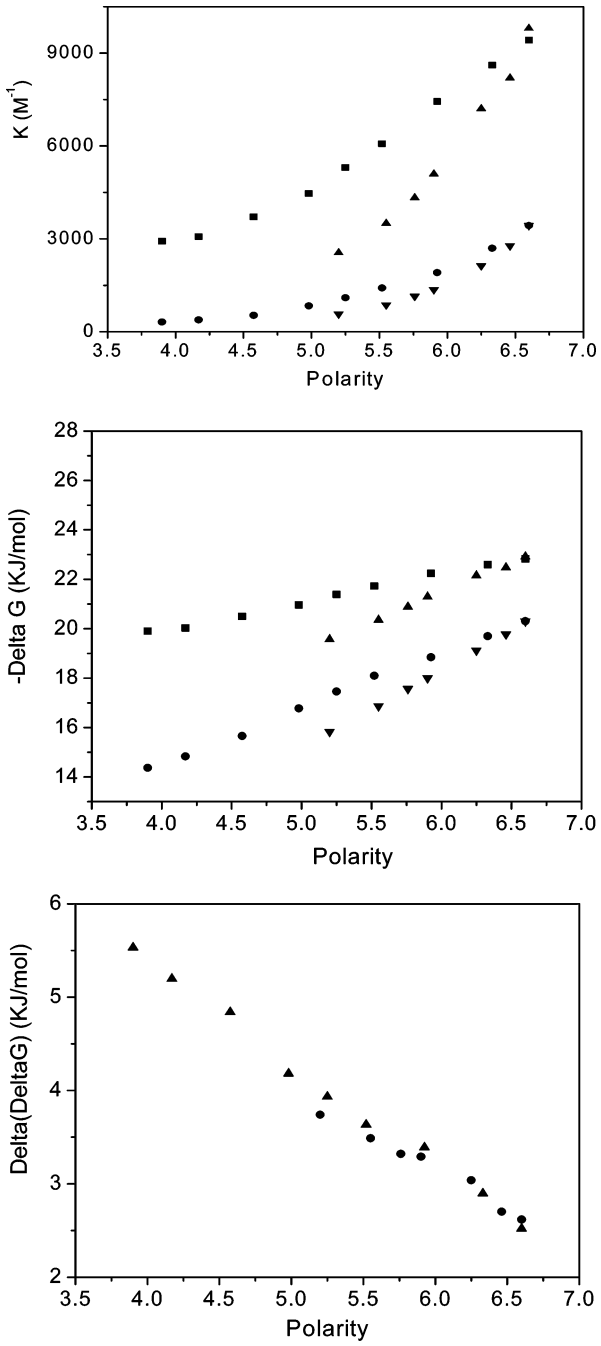

Figure 9. Plots of binding constant (K), free energy of binding (ΔG), and binding energy difference ($\Delta\Delta G$) as a function of solvent polarity for complexes of *t*-BuCQN with *R*-DNB-Leu in methanol:butanol mixtures (●) or in methanol:ethanol mixtures (▼) and of *t*-BuCQN with *S*-DNB-Leu in methanol:butanol mixtures (■) or in methanol: ethanol mixtures (▲).

weaker, it seems, however, responsible for enantiomeric selectivity. This deduction stems from the observation that benzoyl-Leu-OMe cannot bind but that DNB-Leu and DNB-Leu-OMe not only can bind to *t*-BuCQN but also that the bindings exhibit enantiomeric selectivity. Furthermore, the observed enantiomeric selectivity increases as solvent polarity decreases. Therefore, it seems to imply that the closer the electron acceptor dinitrophenyl group is to the electron donor quinoline group, the higher is the enatiomeric selectivity. Specifically, in solvent with high polarity, both donor and acceptor are solvated by solvent molecules, thereby preventing them from being close. As a consequence, the interaction will be weaker and, hence, will lower enantiomeric selectivity. Solvation will be less in a less

TABLE 3: Binding Constants for Complexes of *t*-BuCQN with either (*S*)-DNB-Leu- OMe or (*R*)-DNB-Leu-OMe in Different Solvents

solvent	pol-arity[22,23]	$K_{(S)-\text{DNB}-\text{Leu}-\text{OME}}$ M^{-1}	$K_{(R)-\text{DNB}-\text{Leu}-\text{OME}}$ M^{-1}
50% 1-Cl-butane & CH	0.6	29 ± 6	4 ± 6
1-Cl-butane	1.0	38 ± 3	4 ± 4
di-Cl-methane	3.1	15 ± 3	a
di-Cl-ethane	3.5	2 ± 4	a
1-butanol	3.9	61 ± 5	a
chloroform	4.1	12 ± 1	6 ± 0.6
acetonitrile	6.2	90 ± 8	a
MeOH	6.6	~10	~10

[a] Changes in absorbance were too small to determine binding constants accurately.

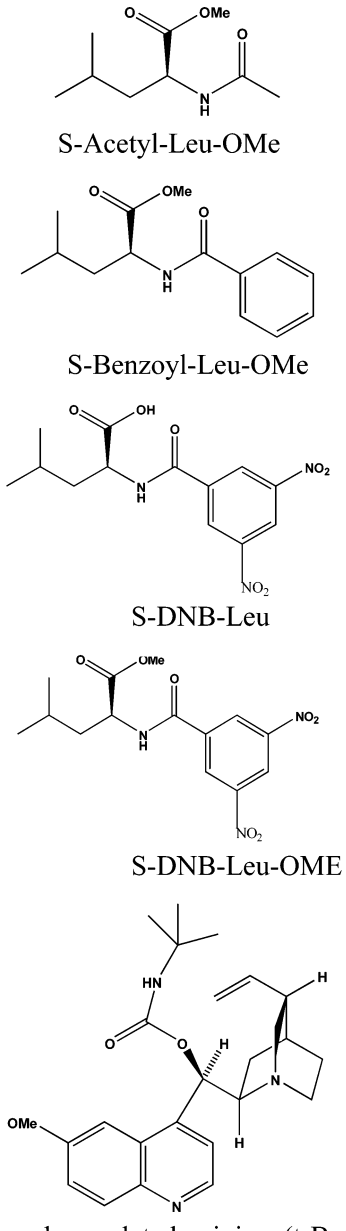

Figure 10. Structure of *tert*-butylcarbamoylquinine (*t*-BuCQN), 3,5-dinitrobenzoyl leucine (DNB), benzolyl-Leu-OMe, and acetyl-Leu-OMe.

polar solvent which, in turn, leads to stronger interaction and higher enantiomeric selectivity. The fact that Ace-Leu-OMe can bind but benzoyl-Leu-OMe cannot bind to *t*-BuCQN indicates that hydrogen bonding between the amide of acetyl-Leu-OMe

(and DNB-Leu) with the carbonyl group of the *t*-BuCQN is essential for the binding. Strongest binding and highest enantiomer selectivity will be observed if all of the three interactions (electrostatic, charge transfer, and hydrogen bonding) are in operation as in the case of DNB-Leu. Experiments are currently in progress to apply and to expand information gained from this study to other systems.

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