Novel Chiral Selector Based on Mefloquine – A Comparative NMR Study to Elucidate Intermolecular Interactions with Acidic Chiral Selectands

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ABSTRACT The synthesis, ab initio calculations, and a comparative nuclear magnetic resonance study of a novel chiral mefloquine-based selector (SO) are presented. On a series of variously *N*-acyl protected leucine selectands (SAs), a feasibility study of mefloquine carbamate as a basic chiral solvating agent, and potential fluorophilic high-performance liquid chromatography selector has been undertaken and evaluated. An analogy is drawn between the new SO and *tert*-butylcarbamoyl quinidine as a reference. *Chirality 00:000-000, 2012.* © 2012 Wiley Periodicals, Inc.

KEY WORDS: NMR; enantiodiscrimination; chiral solvating agents; chiral recognition; carbamoylmefloquine; cinchona alkaloids

INTRODUCTION

Chromatographic separation of racemic mixtures on chiral stationary phases (CSPs) has developed from purely analytical into a widely accepted industrial production tool.[1] The systematic evolution of novel chiral selectors has provided a wide range of CSPs that enable to date the separation of a great variety of racemic mixtures of interest. In the last years, our group has introduced several chiral anion exchanger type SOs and CSPs based on cinchona alkaloids, which show enantioseparation capabilities for a broad range of chiral acidic compounds.[2–4] This successful pattern prompted us to synthesize a novel selector based on mefloquine (MFQ), which is structurally related to the cinchona motif, in particular to quinine (QN) and quinidine (QD). Mefloquine is a well known and most widely used antimalarial drug,[5] and because of its aminoethanol-like constitution, it has been screened for various pharmacological activity.[6,7] As can be seen in Figure 1, the structure of MFQ is derived from cinchona alkaloids. The quinoline ring of MFQ is substituted by two electron-withdrawing groups instead of the electron-donating methoxy group, and the quinuclidine moiety of cinchonans is replaced by a less bulky piperidine ring. Although the strongly deactivating effect of trifluormethyl group is well recognized, in aromatic systems, a strong positive resonance effect was observed.[8] In consequence, this +R effect reduces the π-acidity of the quinoline core of MFQ, which is therefore only weakly π-acidic or "π-neutral". Thus, we assumed that MFQ would sufficiently interact with chiral π-acidic and π-basic analytes (SAs), and the trifluoromethyl groups would provide additional fluorophilic potential in the course of diastereoselective SO–SA intermediate associates primarily driven by ion-pair formation.

Recently, several detailed nuclear magnetic resonance (NMR) studies concerning the specific interactions of π-basic QN- or QD-based chiral selectors (SOs) and negatively charged chiral selectands (SAs) have been published.[9–13] Conceptually, the intermolecular interactions and the molecular recognition principle of the diastereomeric *(R)*-SO-*(R)*-SA and *(R)*-SO-*(S)*-SA associates are based on concerted electrostatic, π–π, steric, and hydrogen bonding interactions leading to well-distinguishable entities. The best separation using a π-basic QN- or QD-based SO in high-performance liquid chromatography (HPLC) was achieved for strongly π-acidic (*S*)-*N*-3,5-dinitrobenzoylleucine and (*R*)-*N*-3,5-dinitrobenzoylleucine, respectively. Following the chemical and functional reciprocity principle, we envisioned that our new selector (9 *S*;10 *R*)-*tert*-butylcarbamoyl-*N*-allyl mefloquine (*t*BuCMFQ) should follow similar intermolecular recognition pathway, as the *tert*-butylcarbamoyl quinidine (*t*BuCQD) type chiral SO but being driven by an ambivalent π–π stacking ability with a π-basic or π-acidic counterpart of the SA. The concept of ambivalence was previously described for nucleophilic–electrophilic potential of organic molecules.[14] Here we refer to the "π-neutral" character of the quinoline core of MFQ, which can thus interact with both π-acidic and π-basic types of SAs.

Using a set of acidic SAs with different electron density distribution on the aromatic moiety, we aimed to prove the validity of this concept. We synthesized a series of reference compounds for the ^{1}H NMR investigations of diastereomer SO–SA mixtures and compared the nonequivalences (differences between the chemical shifts of two enantiomers in the presence of a chiral auxiliary) induced by *t*BuCMFQ and *t*BuCQD. We also investigated the complexation-induced chemical shifts (CISs) of respective SOs in the presence of enantiomerically pure SAs and compared the strength of complexation with results obtained in HPLC measurements. A similar methodology has already been used for comparison of the chiral discriminating properties of cyclodextrine-based selectors in reversed phase (RP) HPLC and NMR.[15]

MATERIALS AND METHODS

General Methods

Nuclear magnetic resonance measurements were performed on Avance DRX NMR spectrometers (Bruker BioSpin, Rheinstetten, Germany)

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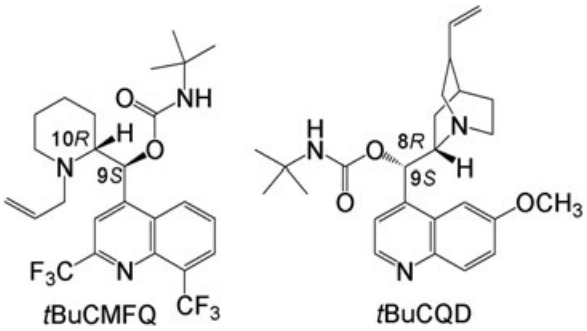

Fig. 1. Selectors and chiral solvating agents, respectively, used in this study.

operating at 600.13 and 400.13 MHz for ^{1}H, 150.92 and 100.62 MHz for ^{13}C, and 564.62 and 376.45 MHz for ^{19}F. Chemical shifts are referenced internal to the residual, nondeuterated solvent signal for ^{1}H ($CDCl_3$: $\delta = 7.26$ ppm; CD_3OD: $\delta = 3.31$ ppm), or to the carbon signal of the solvent for ^{13}C ($CDCl_3$: $\delta = 77.00$ ppm; CD_3OD: $\delta = 49.00$ ppm), or external to $CFCl_3$ ($\delta = 0$ ppm) for ^{19}F. The two-dimensional NMR spectra were obtained by using standard sequences as supplied by the manufacturer. The gradient-enhanced correlation spectroscopy (*ge*-COSY) experiments were carried out with the minimal spectral width required; 128 increments of eight scans and 2 K data points were obtained. The two-dimensional nuclear Overhauser effect spectroscopy (NOESY) spectra were acquired in 2 K data points by using eight scans of 128 increments each, with a mixing time of 0.8 sec. The gradient-enhanced heteronuclear single quantum correlation (*ge*-HSQC) and gradient-enhanced heteronuclear multiple bond correlation (*ge*-HMBC) experiments were performed with the minimum spectral width required in F1 and F2 domain in 2 K data points by using 16 and 64 scans of 128 increments, respectively.

High-performance liquid chromatography measurements were performed on an 1100 Series HPLC system (Agilent Technologies, Waldbronn, Germany) consisting of a solvent degasser, a pump, an autosampler, a column thermostat, and a UV–vis detector. Data acquisition and analysis were accomplished with ChemStation chromatographic data software from Agilent Technologies. Liquid chromatography–mass spectrometry analysis was performed on a 4000 Q TRAP LC/MS/MS system (Applied Biosystems/MDS Sciex, Foster City, USA) equipped with a standard electrospray source and coupled with a 1200 HPLC system (Agilent Technologies). Chromatographic purifications were performed on silica gel Kieselgel 60 (Merck, Darmstadt, Germany). Melting points were measured using a Leica Gallen III system (Reichert). X-ray analysis was performed with graphite-monochromated Mo Kα radiation, $\lambda = 0.71073$ Å at 100(2) K. The single crystal was positioned at 35 mm from the detector, and 691 frames were measured, each for 30 sec over 1° scan width. The data were processed using Bruker's software package.[16] The structure was solved by direct methods and refined by full-matrix least-squares techniques. Nonhydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were placed in geometrically calculated positions and refined as riding atoms in the subsequent least-squares model refinements. The isotropic thermal parameters were estimated to be 1.2 times the values of the equivalent isotropic thermal parameters of the atoms to which hydrogens were bonded. For structure solution SHELXS-97,[17] for refinement SHELXL-97,[18] and for molecular diagram, Crystals (University of Oxford) were used.

Solvents and Reagents

Racemic MFQ hydrochloride was obtained from Kreamer&Martin Pharma Handels – GmbH. Leucine was purchased from Bachem. Dimethylformamide, allyl bromide, *tert*-butylisocyanate, tartaric acid, and substituted benzoic acids were recieved from Sigma-Aldrich. Variously protected leucine derivatives were prepared according to procedures described in the literature.[19,20] Solvents were purchased from VWR Austria; the solvents labeled as dry were distilled from P_2O_5 before use.

Synthetic Procedures

The synthetic route, numbering of prepared compounds and assignment of atoms are depicted in Figures 2 and 3.

***rac*-*N*-Allyl mefloquine (1).** A suspension of MFQ hydrochloride (10.0 g, 24.1 mmol) and K_2CO_3 (3.33 g, 24.1 mmol) in dry dimethylformamide (60 ml) was stirred at room temperature for 15 min. Allyl bromide (2.09 ml, 24.1 mmol) was added and the reaction mixture was stirred at room temperature for 17 h, diluted with water (150 ml) and stirred further for 30 min. The precipitate was filtered, washed with methanol, and dried in vacuum. The crude product was purified by crystallization from methanol. It was obtained 6.94 g (69%) of racemic **1**, mp 125–126 °C, mp lit.[6] 121–123 °C.

(9 *R*;10 *S*)-*N*-Allyl mefloquine (+)-*O*,*O*-diacetyl-L-tartaric acid monoester (3). In an oven-dried and nitrogen-flushed flask, *N*-allyl mefloquine (2.50 g, 5.98 mmol) was dissolved in dry dichloromethane (60 ml). Freshly prepared and dried (+)-*O*,*O*-diacetyl-L-tartaric acid anhydride (2.00 g, 9.34 mmol) was added in the nitrogen counter stream, and the reaction mixture was stirred overnight. It was decomposed with 5% aq. $NaHCO_3$ (100 ml), stirred for 5 min, and acidified with 2 M aq. HCl to pH = 2.5. The layers were separated, and the aqueous layer was extracted with dichloromethane (2×25 ml). The combined organic

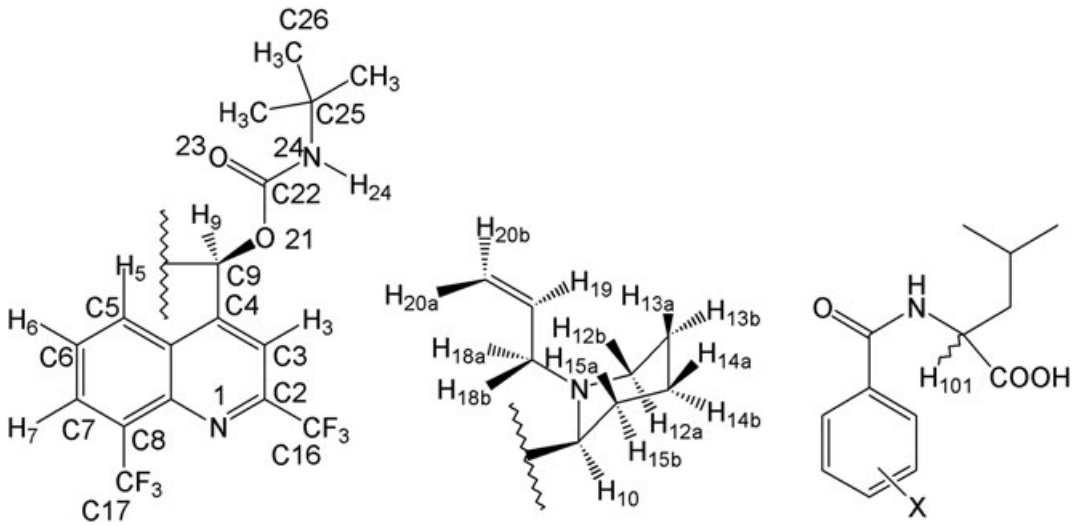

Fig. 2. The assignment of atoms of *t*BuCMFQ and analytes used for NMR measurements.

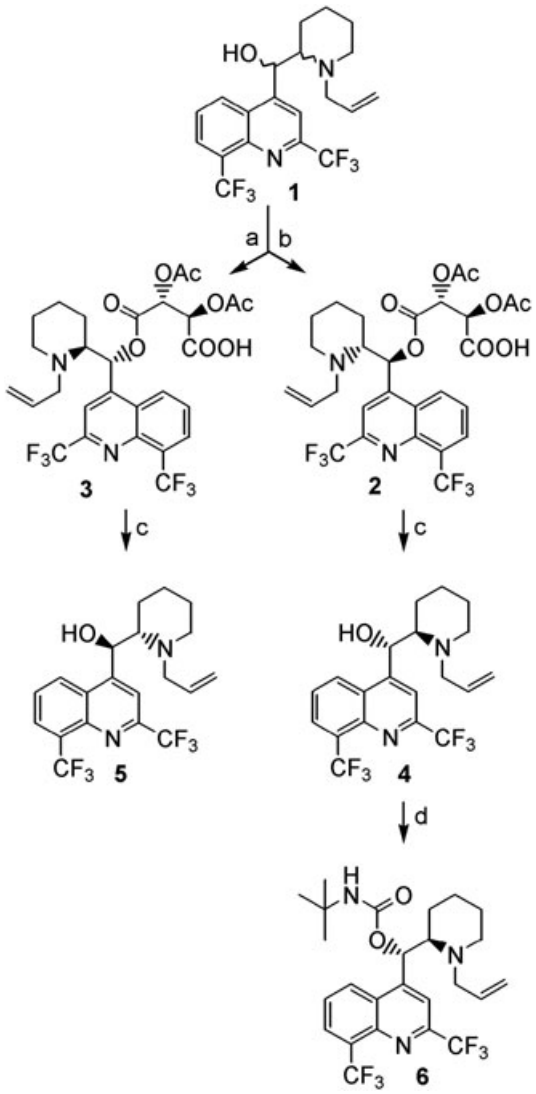

Fig. 3. The synthetic route for the preparation of (9 *S*;10 *R*)-*tert*-butylcarbamoyl-*N*-allyl mefloquine (**6**); (a), (b) (*R*,*R*)-DATAAN, CH_2Cl_2, r.t.; (c) K_2CO_3, CH_2Cl_2/H_2O; (d) *tert*-butylisocyanate, dibutyltin dilaurate, toluene.

solution was washed with water (2 × 25 ml), brine (25 ml), and dried with anhydrous $MgSO_4$. The solvent was evaporated; the crude product was placed in a Soxhlet extractor and extracted with hot toluene (50 ml) for 1 h. The resulting solid was dissolved in dichloromethane, the solvent was evaporated, and the extraction procedure was repeated. The product **2** was harvested as a white crystalline powder, 1.48 g (78%), mp 195.5–197 °C. ^{1}H NMR (400.13 MHz, $CDCl_3$) δ 1.12 (1H, m); 1.33 (1H, m); 1.77 (3H, m); 2.08 (3H, CH_3, s); 2.15 (4H, bs); 2.80 (1H, m); 3.12 (1H, m); 3.96 (1H, bs); 4.24 (1H, m); 4.36 (1H, bs); 5.83 (3H, m); 6.01 (1H, bs); 6.27 (1H, $\underline{CH}CH_2$, m); 6.67 (1H, m); 7.78 (1H, H_6, t, J = 7.8 Hz); 8.12 (1H, H_3, s); 8.21 (1H, s); 8.23 (1H, s). ^{13}C NMR (100.62 MHz, $CDCl_3$) δ 171.4 (CO), 170.8 (CO), 166.6 (CO), 165.9 (CO), 144.4 (C_q), 142.4 (C_q), 129.7 (CH), 128.8 (CH), 126.1 (CH), 125.8 (CH), 125.4 (C_q), 117.0 (CH), 71.7 (CH), 70.9 (CH), 69.4 (CH), 63.4 (CH), 56.2 (CH_2), 55.6 (CH_2), 24.4 (CH_2), 22.9 (CH_2), 22.6 (CH_2), 21.1 (CH_3), 20.4 (CH_3). ^{19}F NMR (376.45 MHz, $CDCl_3$) δ −61.5 (CF_3), −69.3 (CF_3). MS: calculated for $C_{28}H_{28}F_6N_2O_8$ (634.2), found $[M+H^+]$ = 635.3.

(9 *S*;10 *R*)-*N*-Allyl mefloquine (+)-*O*,*O*-diacetyl-L-tartaric acid monoester (2). The combined organic solution from the extraction was evaporated under reduced pressure, and the crude product was purified by column chromatography (eluent dichloromethane/2-propanol, 5/1). It was obtained 1.23 g (65%) of monoester **3**, mp 125.5–127.5 °C. ^{1}H NMR (400.13 MHz, $CDCl_3$) δ 1.10 (1H, m); 1.23 (1H, m); 1.80 (2H, m); 2.02 (4H, bs); 2.12 (4H, bs); 2.72 (1H, m); 3.07 (1H, m); 3.76 (1H, m); 4.06 (2H, m); 5.75 (2H, $\underline{CH_2}CH$, m); 5.85 (1H, d, J = 2.0 Hz); 6.18 (1H, d, J = 2.0 Hz); 6.30 (1H, $\overline{CH}CH_2$, m); 7.01 (1H, H_9, d, J = 2.0 Hz); 7.57 (1H, H_3, s); 7.81 (1H, H_6, J = 8.0 Hz); 8.22 (1H, H_7, s); 8.24 (1H, H_5, s). ^{13}C NMR (100.62 MHz, $CDCl_3$) δ 170.8 (CO), 170.3 (CO), 169.1 (CO), 166.6 (CO), 148.3 (C_q), 148.0 (C_q), 144.8 (C_q), 144.0 (C_q), 130.3 (C_q), 130.0 (C_q), 129.5 (CH), 128.2 ($\underline{CH}CH_2$), 128.1 (CH), 126.3 (CH), 125.6 (C_q), 124.5 ($\underline{CH_2}CH$), 115.0 (CH), 71.9 (CH), 71.6 (CH), 69.7 (CH), 62.9 (CH), 54.8 ($\overline{CH_2}$), 54.2 (CH_2), 24.1 (CH_2), 23.1 (CH_2), 23.0 (CH_2), 20.5 (CH_3), 20.4 (CH_3). ^{19}F NMR (376.45 MHz, $CDCl_3$) δ −60.8 (CF_3), −68.3 (CF_3). MS: calculated $C_{28}H_{28}F_6N_2O_8$ (634.2), found $[M+H]^+$ = 635.1.

(9 *S*;10 *R*)-*N*-Allyl mefloquine (4). To a solution of **2** (0.90 g, 1.42 mmol) in dichloromethane (20 ml), an aqueous solution of K_2CO_3 (15 ml, pH = 10) was added, and the reaction mixture was vigorously stirred at room temperature for 18 h. The separated aqueous solution was extracted with dichloromethane (2 × 40 ml), and the combined organic solution was dried with anhydrous $MgSO_4$. The solvent was evaporated, and the crude product was purified by column chromatography (eluent dichloromethane/methanol, 10/1) to yield 0.53 g (91%) of enantiomerically pure **4**, mp 38–40 °C. ^{1}H NMR (400.13 MHz, CD_3OD, CD_3COOD) δ 1.03 (1H, m); 1.26 (1H, m); 1.68–1.1.88 (4H, m); 3.16 (1H, dt, ^2J = 12.4 Hz, ^3J = 3.8 Hz); 3.58 (1H, m); 3.71 (1H, dt, ^3J = 12.1 Hz, ^3J = 3.1 Hz); 4.17 (1H, dd, ^2J = 14.1 Hz, ^3J = 9.0 Hz); 4.33 (1H, dd, ^2J = 13.8 Hz, ^3J = 5.5 Hz); 5.79 (2H, $\underline{CH_2}$ = CH, m); 6.25 (1 H, CH = CH_2, m); 6.26 (1H, H_9, d, ^3J = 3.4 Hz); 7.94 (1H, H_6, t, ^3J = 8.2 Hz); 8.22 (1H, H_3, s); 8.32 (1H, H_7, d, ^3J = 7.2 Hz); 8.39 (1H, H_5, d, ^3J = 8.6 Hz). ^{13}C NMR (100.62 MHz, CD_3OD, CD_3COOD) δ 151.5 (C_q), 149.4 (C_q), 149.1 (C_q), 144.9 (C_q), 130.6 (CH), 130.2 (C_q), 129.3 (CH), 128.8 (CH), 128.5 (CH), 127.4 (C_q), 126.3 (CH_2CH), 123.6 (C_q), 117.1 (CH), 66.5 (CH), 65.9 (CH), 56.4 (CH_2), 54.0 (CH_2), 24.2 (CH_2), 23.9 (CH_2), 22.7 (CH_2). ^{19}F NMR (376.45 MHz, CD_3OD, CD_3COOD) δ −62.0 (CF_3), −69.8 (CF_3). MS: calculated for $C_{20}H_{20}F_6N_2O$ (418.2), found $[M+H]^+$ = 419.4.

Analogously, (9 *R*;10 *S*)-*N*-allyl mefloquine (**5**) was prepared and 0.50 g (85%) of **5** was obtained, mp 39–40 °C. ^{1}H NMR (400.13 MHz, CD_3OD, CD_3COOD) δ 0.92 (1H, m); 1.15 (1H, m); 1.64–1.76 (4H, m); 2.82 (1H, dt, ^2J = 11.6 Hz, ^3J = 3.9 Hz); 3.27 (1H, m); 3.35 (1H, m); 3.86 (1H, dd, ^2J = 14.2 Hz, ^3J = 8.7 Hz); 4.08 (1H, dd, ^2J = 14.2 Hz, ^3J = 5.5 Hz); 5.61 (2H, $\underline{CH_2}CH$, dd, J_{cisH} = 10.1 Hz, J_{transH} = 17.0 Hz); 6.16 (1H, H_9, d, ^3J = 3.3 Hz); 6.23 (1H, $\overline{CH}CH_2$, m); 7.90 (1H, H_3, t, ^3J = 8.1 Hz); 8.21 (1H. H_3, s); 8.27 (1H, H_7, d, ^3J = 7.3 Hz); 8.42 (1H, H_5, d, ^3J = 8.5 Hz). ^{13}C NMR (100.62 MHz, CD_3OD, CD_3COOD) δ 153.0 (C_q), 149.3 (C_q), 148.9 (C_q), 144.8 (C_q), 132.0 ($\underline{CH}CH_2$), 130.4 (CH), 129.1 (CH), 128.9 (CH), 127.7 (C_q), 126.4 (C_q), 123.7 (C_q); 123.0 ($\underline{CH_2}CH$), 117.0 (CH), 67.4 (CH), 65.3 (CH), 56.9 (CH_2), 54.0 (CH_2), 24.7 (CH_2), 24.5 (CH_2), 23.6 (CH_2). ^{19}F NMR (376.45 MHz, CD_3OD, CD_3COOD) δ −62.0 (CF_3), −69.8 (CF_3). MS: calculated for $C_{20}H_{20}F_6N_2O$ (418.2), found $[M+H]^+$ = 419.3.

(9 *S*;10 *R*)-*tert*-Butylcarbamoyl-*N*-allyl mefloquine (6). To a solution of **4** (0.50 g, 1.20 mmol) and *tert*-butylisocyanate (0.34 ml, 2.99 mmol) in dry toluene (25 ml), dibutyltin dilaurate (0.02 ml) was added and the reaction mixture was heated to 90 °C for 24 h. The solvent was removed, the crude product was dried in vacuum at 50 °C, and the reaction procedure was repeated under the conditions given previously. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography (eluent CH_2Cl_2/MeOH, 20/1). It was obtained 0.52 g (84%) of compound **6**, mp 49.5–50.5 °C. ^{1}H NMR (600.13 MHz, CD_3OD) δ 1.10 (1H, H_{15a}, m) 1.12 (1H, H_{14b}, m); 1.29 (9H, $(CH_3)_3$, s); 1.59 (2H, $H_{13a,b}$, m); 1.66 (1H, H_{15a}, m); 1.71 (1H, H_{14a}, m) 2,50 (1H, H_{12a}, dt, ^2J = 12.1 Hz, ^3J = 13.1 Hz); 2.89 (1H, H_{10}, m); 3.10 (1H, H_{12b}, dt, ^2J = 12.1 Hz, ^3J = 3.9 Hz); 3.44 (1H, H_{18b}, dd, ^2J = 14.2 Hz, ^3J = 8.2 Hz); 3.69 (1H, H_{18a}, dd, ^2J = 14.2 Hz, ^3J = 4.6 Hz); 5.38 (1H, H_{20b}, d, ^3J = 10.1 Hz); 5.44 (1H, H_{20a}, d, ^3J = 17.2 Hz); 6.09 (1H, H_{19}, dddd, $^3J_{19\text{-}20a}$ = 17.2, $^3J_{19\text{-}20b}$ = 10.1, $^3J_{19\text{-}18b}$ = 8.2, $^3J_{19\text{-}18a}$ = 4.6); 6.86 (1H, H_9, d, ^3J = 3.8 Hz); 7.90 (1H, H_6, t, $^3J_{6\text{-}5}$ = 8.5 Hz, $^3J_{6\text{-}7}$ = 7.3 Hz); 7.94 (1H, H_3, s); 8.29 (1H, H_7, d, ^3J = 7.3 Hz); 8.52 (1H, H_5, d, ^3J = 8.5 Hz). ^{13}C NMR (150.92 MHz, CD_3OD) δ 155.6 (CO); 151.3 (C_4); 148.8 (C_2); 144.9 (C_{8a});

135.3 (C_{19}); 130.7 (C_7); 130.2 (C_8); 129.4 (C_5); 129.0 (C_6); 127.7 (C_{4a}); 125.0 (C_{17}); 122.7 (C_{16}); 120.0 (C_{20}); 116.4 (C_3); 70.4 (C_9); 63.5 (C_{10}); 57.8 (C_{18}); 54.2 (C_{12}); 51.3 (C_{25}); 28.9 (C_{26}); 25.8 (C_{15}); 25.6 (C_{13}); 24.6 (C_{14}). ^{19}F NMR (564.62 MHz, CD_3OD): −62.1(CF_3–C_{17}); −70.0(CF_3–C_{16}). MS: calculated for $C_{25}H_{29}F_6N_3O_2$ (517.2), found $[M+H]^+ = 518.0$.

(*R*)-*N*-3,5-Bis(trifluormethyl)benzoylleucine (R-BTFMB-Leu). To a solution of (*R*)-leucine (0.13 g; 0.97 mmol) and $(iPr)_2NEt$ (0.17 ml; 0.97 mmol) in 50% aqueous acetonitrile (20 ml), *O*-succinimidyl-3,5-bis (trifluoromethyl)benzoate (0.30 g; 0.97 mmol) was added portionwise. The reaction mixture was stirred and heated to 40 °C over night, acidified with 2 M aq. HCl to pH = 2, diluted with a saturated solution of NaCl (100 ml) and extracted with ethyl acetate (10 × 20 ml). The combined organic solution was dried with anhydrous $MgSO_4$. The solvent was evaporated, and the crude product (**R-BTFMB-Leu**) was purified by column chromatography (eluent dichloromethane/methanol, 5/1). It was obtained 0.21 g (66%) of white crystalline product, m.p. 169.5–172.5 °C. ^{1}H NMR (400.13 MHz, CD_3OD) δ 1.00 (6H, 2 × CH_3, m); 1.79 (3H, CH_2, CH, m); 4.68 (1H, H_{101}, m), 8.16 (1H, CH, s), 8.48 (2H, 2 × CH_2, s). ^{13}C NMR (100.62 MHz, CD_3OD) δ 176.2 (C = O); 166.9 (C = O); 137.9 (C_q); 129.2 (CH); 126.0 (CH); 123.3 (C_q); 53.3 (CH); 41.4 (CH_2); 26.4 (CH); 23.4 (CH_3); 21.7 (CH_3). ^{19}F NMR (376.45 MHz, CD_3OD) δ −64.9 (2 × CF_3). MS: calculated for $C_{15}H_{15}F_6NO_3$ (371.1), found $[M+H]^+ = 372.2$.

Analogously, (*S*)-*N*-3,5-bis(trifluormethyl)benzoylleucine **(S-BTFMB-Leu)** was prepared, mp 168–171 °C. ^{1}H NMR (400.13 MHz, CD_3OD) δ 0.96 (6H, 2 × CH_3, m); 1.80 (3H, m); 4.68 (1H, H_{101}, m); 8.15 (1H, CH, s); 8.46 (2H, 2 × CH, s). ^{13}C NMR (100.62 MHz, CD_3OD) δ 176.0 (CO); 166.8 (CO); 137.8 (C_q); 129.2 (CH); 126.0 (CH); 123.3 (C_q); 53.9 (CH); 41.4 (CH_2); 26.3 (CH); 23.4 (CH_3); 21.6 (CH_3). ^{19}F NMR (376.45 MHz, CD_3OD) δ −64.8 (2 × CF_3). MS: calculated for $C_{15}H_{15}F_6NO_3$ (371.1), found $[M+H]^+ = 372.0$.

RESULTS AND DISCUSSION

Synthesis

The separation of (*rac*)-**1** by using (+)-*O*,*O*-diacetyl-L-tartaric acid anhydride was performed using a method described in the literature.[21] It was monitored with RP HPLC, using a Kinetex RP C-18 (150 × 4.6 mm, 5 μm) column. Each diastereomer was obtained in diastereomeric excess > 99%.

Assignment of Absolute Configuration of Mefloquine Derivatives

Although the absolute configuration of MFQ is described in the literature,[22,23] the data correlating the elution order of the respective enantiomers with their absolute configuration are not available for the *N*-allyl derivative. Therefore, we decided to use the similarity between cinchona alkaloids and MFQ. We employed (+)-*O*,*O*-dibenzoyl-L-tartaric anhydride derivatized QN and QD as reference compounds in an RP HPLC measurement (Fig. 4). With these results, we were able to assign indirectly via a chromatographic elution order of the diastereomers the absolute configuration of the less retained diastereomer as (9 *S*;10 *R*)-*N*-allyl mefloquine (+)-*O*,*O*-dibenzoyl-L-tartric acid monoester and of the more retained diastereomer as (9 *R*;10 *S*)-*N*-allyl mefloquine (+)-*O*,*O*-dibenzoyl-L-tartric acid monoester. We also prepared a cocrystal of (9 *R*;10 *S*)-*N*-allyl mefloquine with monomethyl (+)-*O*,*O*-diacetyl-L-tartrate and performed an X-ray analysis (Fig. 5) that confirmed the result obtained by the HPLC measurement.

Ab initio Calculations

Having the confirmed starting structure of (9 *R*;10 *S*)-*N*-allyl mefloquine, we decided to perform the ab initio calculations for (9 *R*;10 *S*)-*tert*-butylcarbamoyl-*N*-allyl mefloquine. The structure of 9-*O*-*tert*-butylcarbamoyl QN was confirmed in a previous study,[9] in which it was shown that QN-based selector exhibited remarkable complexation properties to (*S*)-*N*-3,5-dinitrobenzoylleucine. This behavior is strongly influenced by π−π stacking of the aromatic moieties of respective compounds. To support this assumption and the reciprocity principle with MFQ-based selector, we calculated electron density of selected SO–SA couples (Fig. 6). Optimization to minimum energy was carried out in Gaussian 03 on DFT B3LYP 6–31 g(d) level.[24] Because protonation realizes selectively on the piperidine or quinuclidine ring of the

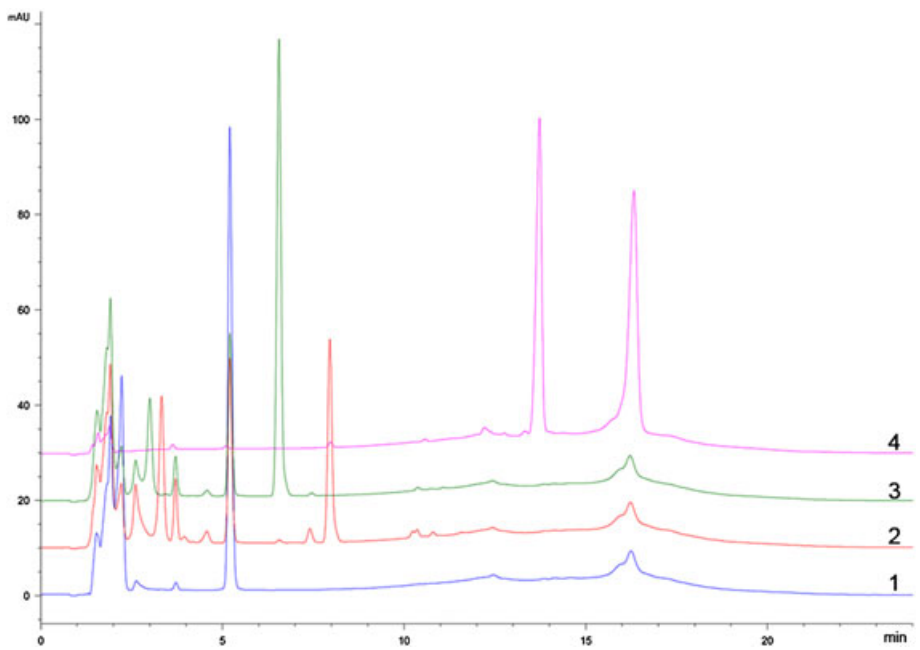

Fig. 4. Reversed phase high-performance liquid chromatography measurement of (+)-*O*,*O*-dibenzoyl-L-tartaric monoesters: hydrolyzed (+)-*O*,*O*-dibenzoyl-L-tartaric anhydride (1) and respective monoesters of quinine (2), quinidine (3), and *rac*-*N*-allyl-mefloquine (4).

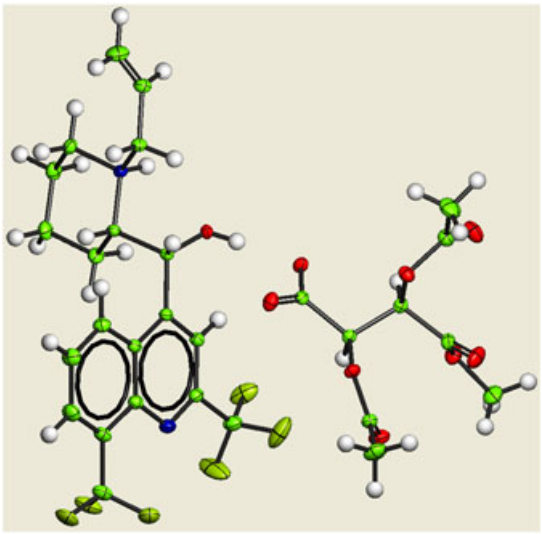

Fig. 5. X-ray structure of a cocrystal of (9 *R*;10 *S*)-*N*-allyl mefloquine with (+)-*O*,*O*-diacetyl-L-tartaric acid monomethyl ester (CCDC 850684).

respective selectors (*vide infra*), we assume that the aromatic core would be affected by this *N*-protonation to a much lesser extent than the aliphatic part of the molecule, and thus, free-base forms of the selectors were used for calculations.

As can be seen in Figure 6, the π–π stacking between QN selector and (*S*)-DNB-Leu is obviously favored because a strong π-base (SO) interacts with a strong π-acid (SA). As envisioned, the situation is different for MFQ selector, which should be a weak π-acid. The two trifluoromethyl electron-withdrawing groups represent the electron-rich part of the molecule, whereas the aromatic system is neither electron-rich nor electron-deficient. The selectand, which we designed as the strong π-base for effective π–π interaction with MFQ, is essentially π-basic but also spatially demanding. Consequently, this feature can negatively influence its π–π stacking abilities.

HPLC Determination of the Differential Free Binding Energies $\Delta_{R,S}$ (ΔG)

In a preceding study performed in our work group,[25] we demonstrated that the enantioselectivity of cinchona carbamates is not significantly influenced by immobilization on modified silica. Therefore, nonselective retention increments or adverse effects caused by immobilization can be neglected, and the calculated $\Delta_{R,S}$ (ΔG) data can be considered as good estimates for $\Delta_{R,S}$ (ΔG) values. Using the QD-AX column (commercially available *t*BuCQD), we measured different SAs (Fig. 7) in racemic as well as enantiomerically pure forms. From the respective α-values, the corresponding differential free binding energies were calculated (Table 1).

As expected, π-acidic SAs were better resolved than the π-basic SAs. It is also obvious (Table 1) that not only π–π but also spatial interactions play an important part in the molecular recognition process. This is clearly documented by the so-called "Linker-Leu" derivative. It is not as π-acidic as DNB-Leu or DCB-Leu, but it is still very well enantioseparated on a π-basic QD-AX. Surprisingly high α-value was obtained for TMB-Leu, although it was less retained, which we designed as a strongly π-basic SA for the MFQ selector. As shown in Figure 6, TMB-Leu is a very spatially demanding molecule, and thus, π–π interactions are suppressed; however, no change of elution order was noticed. The stericaly preferred enantiomer can form with the selector a strong complex based on London dispersion forces, which leads to an effective enantioseparation with α-value of 5.5. Such interactions are not affordable to Bz-Leu, which can be, in addition, considered as a π-neutral molecule. Although it possesses a highly π-acidic aromatic core, the lowest α-value was found for the resolution of PFB-Leu. Overall, it was also the weakly retained compound, similar to (*S*)-BTFMB-Leu, (*S*)-Bz-Leu and (*S*)-TMB-Leu.

NMR Spectroscopy

A full characterization of the MFQ selector **6** (Fig. 3) as well as the selectands (Fig. 7) was performed by comparative analysis of two-dimensional spectra of homonuclear and heteronuclear correlations. On the basis of a previous study,[9] the conformation of *t*BuCMFQ can be described as anti-open-like. Analogously to anti-open conformation of cinchonans, the most pronounced nuclear Overhauser effects were found between protons H_9–H_{10} and H_5–H_{10}. Because of higher flexibility and a lower space filling ability of the piperidine ring, no other correlation between an aromatic proton and the aliphatic ring was obtained.

Similarly to cinchona alkaloids, *tert*-butylcarbamoyl-*N*-allyl mefloquine (SO1) is a multifunctional compound able to form higher order aggregates.[26] To eliminate this phenomenon, we performed all our measurements with diluted samples (20 mM) in methanol-d_4. This solvent should simulate the HPLC measurement conditions in a polar organic mode. The binding stoichiometry between *t*BuCMFQ and both enantiomers of TMB-Leu as the potentially best π-basic selectand was determined by a continuous variation titration protocol.[27,28] The most pronounced CISs were found for the benzylic proton H_9 with the maximum value at TMB-Leu molfraction of 0.5 for the more stable *t*BuCMFQ-(*R*)-TMB-Leu and the less stable *t*BuCMFQ-(*S*)-TMB-Leu complex. The corresponding Job plot is depicted in Figure 8.

Such behavior is typical for a 1:1 complexation and indicates that, in the first place, the piperidine nitrogen is involved in the formation of a salt bridge (ion pair). This hypothesis is further supported by pronounced CISs of the protons H_{10}, H_{18}, and H_{12} that are located in the vicinity of piperidine nitrogen.

A series of equimolar mixtures of stronger complexed pure (*R*)-enantiomers of SAs with each SO were prepared. The

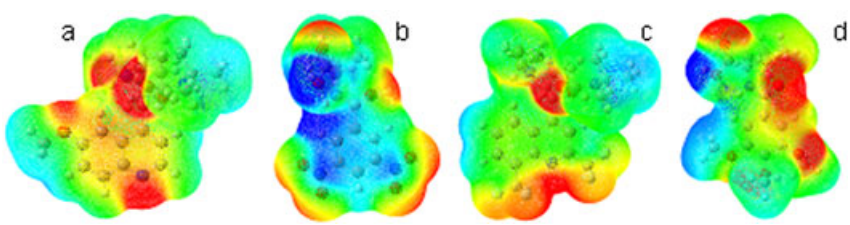

Fig. 6. Electrostatic potential-derived charges – total electron density of (**a**) *t*BuCQN selector, (**b**) (*S*)-DNB-Leu, (**c**) (9 *R*;10 *S*)-*t*BuCMFQ, (**d**) (*S*)-TMB-Leu. Color code: red, the highest electron density; blue, the lowest electron density. [Color figure can be viewed in the online version, which is available at wileyonlinelibrary.com.]

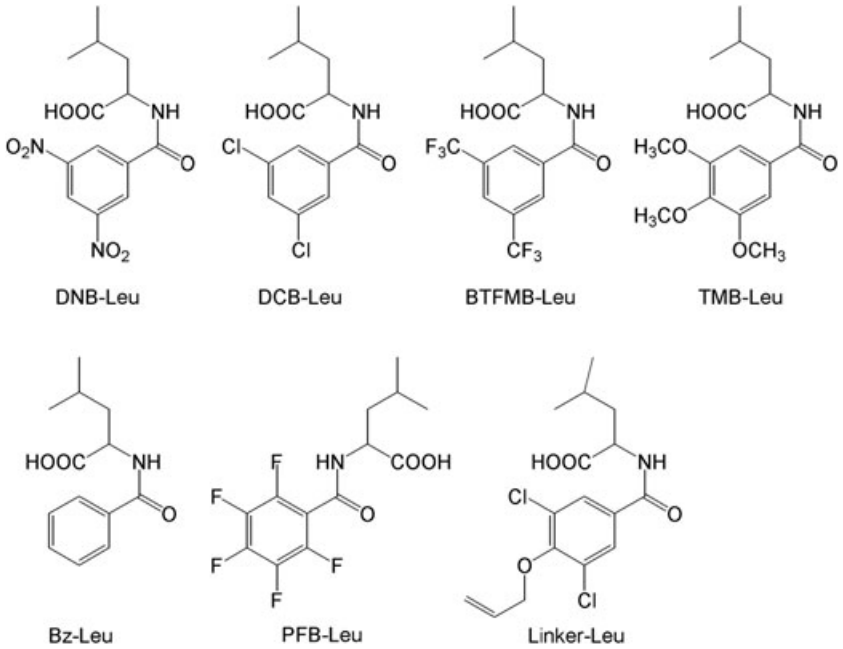

Fig. 7. The list of analytes used in the study.

TABLE 1. Differential free binding energies. Chiral stationary phase: QD-AX. Mobile phase: methanol 98%, acetic acid 2%, ammonium acetate 0.5%. All measurements were carried out at 23 °C

	t_0	t_R	k	α	ΔG[kJ/mol]
(*R*)-DNB-Leu	1.86	51.77	26.85	11.00	−5.90
(*S*)-DNB-Leu	1.86	6.40	2.44		
(*R*)-LINKER-Leu	1.86	27.64	13.87	9.35	−5.50
(*S*)-LINKER-Leu	1.86	4.62	1.48		
(*R*)-BTFMB-Leu	1.86	12.46	5.70	9.04	−5.42
(*S*)-BTFMB-Leu	1.86	3.03	0.63		
(*R*)-DCB-Leu	1.86	39.30	20.14	6.69	−4.68
(*S*)-DCB-Leu	1.86	7.62	3.01		
(*R*)-TMB-Leu	1.86	12.20	5.56	5.49	−4.19
(*S*)-TMB-Leu	1.86	3.74	1.01		
(*R*)-Bz-Leu	1.86	6.54	2.52	2.52	−2.28
(*S*)-Bz-Leu	1.86	3.73	1.00		
(*R*)-PFB-Leu	1.86	4.50	1.69	1.76	−1.39
(*S*)-PFB-Leu	1.86	3.64	0.96		

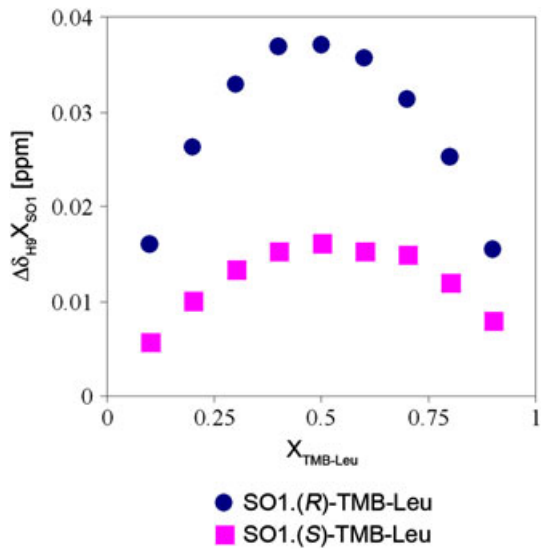

Fig. 8. Job plot for the diastereomeric complexes *t*BuCMFQ-(*R*)-TMB-Leu and *t*BuCMFQ-(*S*)-TMB-Leu. The total concentration was 20 mM.

recorded ^{1}H NMR spectra were compared with that of pure 10 mM chiral substrates. The diagnostic protons H_9 of *t*BuCMFQ and *t*BuCQD exhibited the most pronounced shifts and also clearly showed the difference between the studied selectors.

We found a similar trend for the concentration-related CISs of *t*BuCQD with (*R*)-SAs as we observed in HPLC experiments (Fig. 9). With the exception of (*R*)-Bz-Leu and (*R*)-PFB-Leu, all the SAs were complexed with a similar strength. In this case, the complex of (*R*)-TMB-Leu with the selector appears to be the strongest one. This again indicates that spatial arrangement of SA–SO pair may be even more important than reciprocal π–π interactions.

A similar trend was found also for the complexation of *t*BuCMFQ with the SAs (Fig. 10). Analogous complexation was found for very strong π-acid ((*R*)-DNB-Leu) and weaker π-acid ((*R*)-Linker-Leu) as well as for the pair of π-acidic (*R*)-BTFMB-Leu and π-basic (*R*)-TMB-Leu. (*R*)-PFB-Leu was again the least complexed SA. Besides the homologues trends of the two selectors for the given SAs, it should be mentioned that the performance in complexation of the strongly bound (*R*)-SAs was one order of magnitude different for each other. This is remarkable and may be reflected by the chromatographic behavior as well.

To directly compare the performance of the novel MFQ selector, we prepared a series of racemic as well as enantiomer-enriched mixtures of SAs with *t*BuCQD and *t*BuCMFQ (Table 2). The establishment of binding isotherms would be necessary for calculation of relative free energies of binding from NMR measurements. As an approximation, we used differences in CISs obtained at a single concentration

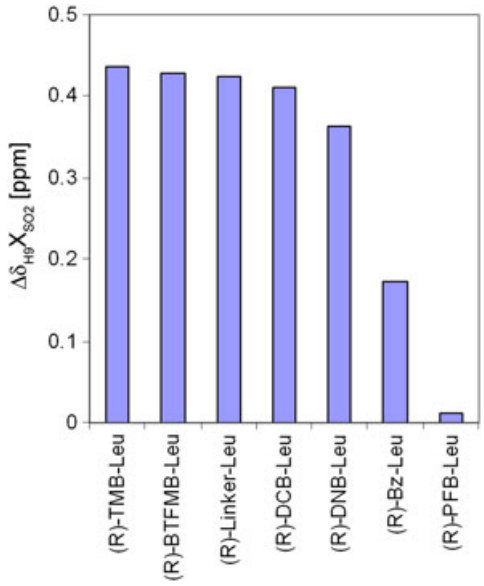

Fig. 9. Concentration dependent CISs of H_9 of *t*BuCQD (SO2) with *N*-acyl protected (*R*)-leucines.

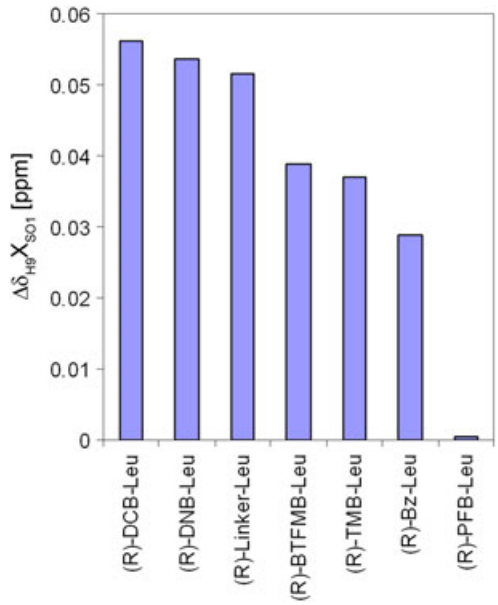

Fig. 10. Concentration dependent CISs of H_9 of *t*BuCMFQ (SO1) with *N*-acyl protected (*R*)-leucines.

corresponding to 1:1 SO–SA complex and relate them to α-values obtained in HPLC. The difference of CISs of respective enantiomers found for the racemic SAs with *t*BuCQD corresponded very well to the data obtained with HPLC (see Table 1). Such behavior is in accordance with results reported in the literature.[15]

The nonequivalence values obtained for *t*BuCMFQ are, in general, much lower than for *t*BuCQD. In addition, the enantioseparation was observed mainly for π-acidic SAs. It can be seen (Table 2) that there was no distinction for π-basic TMB-Leu, although the pure enantiomers were complexed with different strength. As we stated previously, TMB-Leu is most probably spatially demanding, and any typical π–π type of interaction is therefore problematic.

TABLE 2. ^{1}H NMR (600 MHz, CD_3OD, 25 °C) CISs and nonequivalences of SAs (10 mM) in the presence of equimolar amounts of *t*BuCMFQ and *t*BuCQD

	MFQ-AX		QD-AX	
SA	H_{101}	δ	H_{101}	δ
DNB-Leu	−0.01(*R*)	0.017	0.10(*R*)	0.146
	−0.03(*S*)		−0.05(S)	
Linker-Leu	0.10(*R*)	0.07	0.20(*R*)	0.132
	0.09(*S*)		0.07(*S*)	
BTFMB-Leu	−0.01(*R*)	0.011	0.09(*R*)	0.129
	−0.03(*S*)		−0.04(*S*)	
DCB-Leu	0(*R*)	0	0.08(*R*)	0.128
	0(*S*)		−0.04(*S*)	
TMB-Leu	0(*R*)	0	0.09(*R*)	0.103
	0(*S*)		−0.01(*S*)	
Bz-Leu	0(*R*)	0	0.01(*R*)	0.048
	0(*S*)		−0.04(*S*)	
PFB-Leu	0.01(*R*)	0.017	0(*R*)	0
	0(*S*)		0(*S*)	

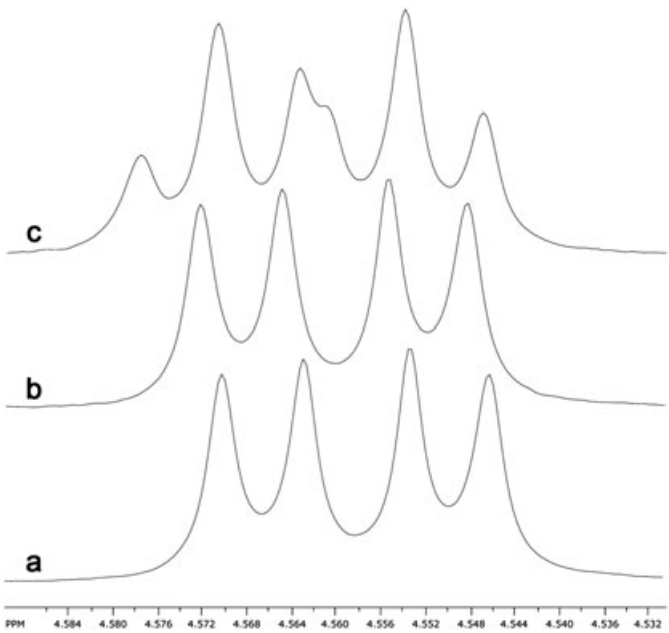

Fig. 11. ^{1}H NMR resonances of H_{101} of PFB-Leu: (a) without a selector, (b) with *t*BuCQD, (c) with *t*BuCMFQ.

For the MFQ selector, we observed by NMR a satisfactory distinction factor of 0.017 with small low-frequency shifts for DNB-Leu. Similar signal separation with δ of 0.011 was found for BTFMB-Leu. It has to be noted that a distinction value of 0.017 was found for PFB-Leu; while using *t*BuCQD, no enantiodiscrimination was observed (Fig. 11). That means that the trifluoromethyl groups of MFQ are capable to support the recognition of PFB-Leu, which is most probably realized by SO–SA fluorophilic interactions. Fluorophilicity is defined as the natural logarithm of the perfluoro(methylcyclohexane)/toluene partition coefficient (P).[29,30] We use this term to point out that a molecule with a certain amount of fluorine atoms may express enhanced affinity to another fluorine-containing molecule.

As it has been shown, the HPLC separation of PFB-Leu with QD-AX is satisfactory but not excellent. However, under the

conditions of the NMR experiment, we did not observe any distinction. The resolution found in NMR measurements with *t*BuCMFQ as the chiral discriminating agent supports our assumption that fluorophilic interactions of the trifluoromethyl groups of MFQ enter the process of chiral recognition. Assuming that we would obtain analogous selector coverage of *t*BuCMFQ as for *t*BuCQD on the respective CSPs, we should be able to relate the nonequivalencies obtained by NMR measurements to the performance of the MFQ selector in HPLC. In such a case, we can expect separation of PFB-Leu with $\alpha > 1.76$. That means that *t*BuCMFQ has a potential as a selector in HPLC for separation of polyfluorinated compounds.

CONCLUSION

We synthesized a novel chiral selector based on MFQ and evaluated its properties as a chiral discriminating agent for *N*-acyl-protected amino acids. Although this selector does not exhibit expected ambivalent complexation and separation abilities, it showed a certain affinity to polyfluorinated compounds. We documented this behavior on a sufficient NMR resolution of *rac*-*N*-pentafluorobenzoylleucine. This feature requires further investigations with a polyfluorinated version of the *N*-allyl mefloquine carbamate type selector, which could provide improved enantiodiscrimination of chiral polyfluorinated and perfluorinated molecules.

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