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A New HPLC Chiral Stationary Phase for the Direct Resolution of Racemic Quinolone Antibacterials Containing a Primary Amino Group

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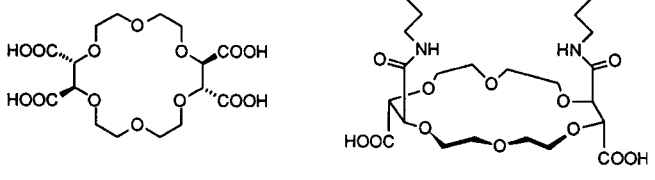
Since norfloxacin was reported to show potent antibacterial activity,[1] a number of new quinolone antibacterials such as ofloxacin, enoxacin, ciprofloxacin, lomefloxacin and fleroxacin have been developed. Among others, ofloxacin is chiral and it is quite interesting to note that the (−)-S-enantiomer is more active than the (+)-R-enantiomer or the racemic form.[2] The effort to develop more potent quinolone antibacterials is still going on and various quinolones have been prepared.[3] Some of them are also chiral. Consequently, during the process of developing new quinolone antibacterial agents, the establishment of analytical techniques that distinguish between two enantiomers is essential in order to meet the government regulations such as US FDA's guide lines for the development of new stereoisomeric drugs.[4] In this aspect, liquid chromatographic direct separation of enantiomers on chiral stationary phases (CSPs) might be the choice because this technique has been known as one of the most convenient and accurate means of determining the enantiomeric composition of chiral compounds.[5] In this study, we wish to report that a new HPLC chiral stationary phase (CSP) derived from (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** can be successfully employed in resolving various investigational racemic quinolones containing a primary amino group. Previously compound **1** has been utilized in resolving primary amino compounds by capillary zone electrophoresis.[6] However, to the best of our knowledge, the use of compound **1** bonded to silica gel as an HPLC CSP has not been reported.

A new CSP (CSP **2**) used in this study was prepared by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** (available from Aldrich) to amino propyl silica gel *via* simple two step procedure. (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid **1** was first converted into its dianhydride by treating with acetyl chloride *via* the known procedure.[7] And then, the dianhydride compound was treated in dry methylene chloride at 0 °C under an argon atmosphere for 2 days with triethylamine and aminopropyl silica gel (particle size: 5 μm, available from Rainin) which was dried in advance by azeotropic removal of water in refluxing benzene. The modified silica gel (CSP **2**) was washed with methanol, water, 1 N HCl solution, water, methanol, dichloromethane and hexane and then dried under high vacuum.[8] The structure of CSP **2** is believed to be *syn*-diamide form based on the previous study concerning the stereoselective *syn*-opening of the dianhydride by primary amino compound in the presence of triethylamine.[9] CSP **2** thus prepared was slurried in methanol and then packed into a 150 mm × 4.6 mm I.D. stainless-steel HPLC column using a conventional slurry packing method with an Alltech slurry packer.



1 **CSP 2**

CSP **2** was successfully employed in resolving various investigational racemic quinolones **3** containing a primary amino group.[10] The representative chromatograms are shown in Figure 1 and the resolution results are summarized in Table 1. As shown in Table 1, eight quinolones (**3a-h**) based on 4(1H)-quinolinone-3-carboxylic acid and four quinolones (**3i-l**) based on 4-oxo-1,8-naphthyridine-3-carboxylic acid are resolved with reasonable separation factors. Elution orders were determined only for two configurationally known quinolones (**3e** and **j**), the (R)-enantiomer being retained longer. For the quinolone (**3l**) containing two chiral centers, all of the four stereoisomers are separated as shown in Figure 1b and in Table 1. However, at the present stage, we were not able to assign which peak corresponds to which isomer be-

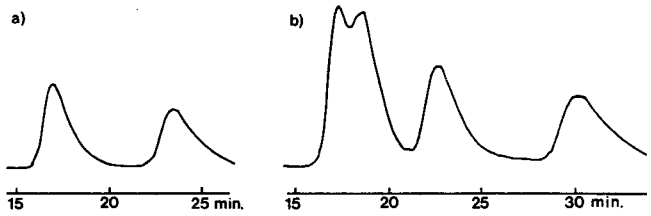
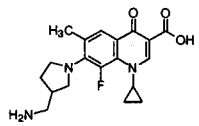
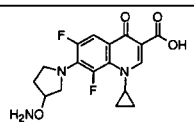
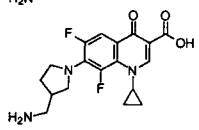
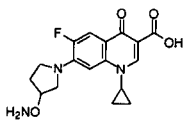
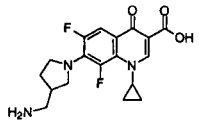
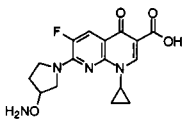
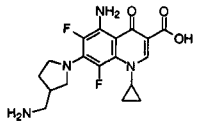
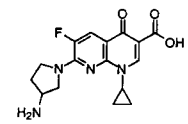
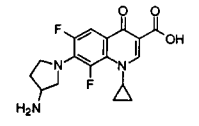
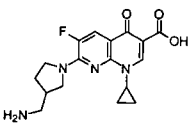
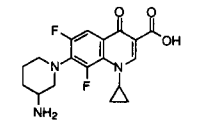
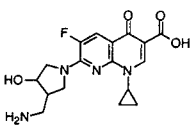


Figure 1. Resolution of (a) quinolone **3h** and (b) quinolone **3l** on CSP **2**. Chromatography was performed with an HPLC system consisting of a Waters Model 510 pump, Waters Model U6k Liquid Chromatographic Injector, Waters Model 441 Absorbance Detector and Waters Model 740 data Module Recorder. Chromatograms were obtained by using 80% methanol in water containing H_2SO_4 (10 mM, pH=1.6) as a mobile phase with flow rate of 1.2 mL/min at 20 °C.

Table 1. Resolution of various quinolones containing a primary amino group on CSP **2**[a]

Quinolone	Data[b]	Quinolone	Data[b]
3a 	k_1'=11.04 k_2'=12.73 α=1.15 R_S=0.71	**3g** 	k_1'=12.27 k_2'=15.64 α=1.27 R_S=1.65
3b 	k_1'=14.28 k_2'=16.44 α=1.15 R_S=0.73	**3h** 	k_1'=13.27 k_2'=18.61 α=1.40 R_S=2.16
3c 	k_1'=12.50 k_2'=14.44 α=1.16 R_S=0.87	**3i** 	k_1'=12.33 k_2'=17.68 α=1.43 R_S=2.20
3d 	k_1'=16.26 k_2'=18.31 α=1.13 R_S=0.77	**3j** 	k_1'=14.01 k_2'=14.76 (R) second α=1.05 R_S=0.22
3e 	k_1'=9.50 k_2'=10.94 (R) second α=1.15 R_S=0.71	**3k** 	k_1'=14.43 k_2'=17.20 α=1.19 R_S=0.81
3f 	k_1'=0.43 k_2'=1.08 α=1.24 R_S=0.81	**3l** 	k_1'=13.53 k_2'=14.58 k_3'=17.95 k_4'=24.21

[a] For the chromatographic conditions, see the caption of Figure 1. [b] Chromatographic resolution data: k_1'=capacity factor for the first eluted enantiomer, k_2'=capacity factor for the second eluted enantiomer, k_3'=capacity factor for the third eluted enantiomer, k_4'= capacity factor for the fourth eluted enantiomer, α=separation factor, R_S=resolution factor.

cause the peak size of the four stereoisomers are quite similar and consequently the separation factors for the two enantiomers are not calculated. We also tried to resolve racemic quinolones containing a secondary amino group, but it was not successful.

The chiral recognition mechanism is not clear yet. However, complexation of the primary ammonium group formed from the primary amino group of quinolones and the acidic modifier in the mobile phase inside the cavity of 18-crown-6 ring of the CSP is believed to be essential for chiral recognition.[11] In addition, the two carboxylic acid groups of the CSP might interact with the other polar substituents of quinolones for chiral recognition. But, the details of the chiral recognition mechanism need further study and are underway in our laboratory.

In conclusion, CSP **2** derived from readily available (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** *via* the two step procedure was successful in separating the two enantiomers of racemic quinolones containing a primary amino group. CSP **2** is expected to be also useful in resolving other class of racemic compounds containing a primary amino group. The study for the use of CSP **2** in the resolution of other racemic primary amino compounds is underway in our laboratory.

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8. The more detailed synthetic procedure will be reported elsewhere. Elemental analysis of the modified silica gel, CSP **2** (found: C, 4.74%; H, 0.77%; N, 0.35%) showed the increase in the content of carbon and the decrease in the content of nitrogen compared to the contents of carbon and nitrogen of original aminopropyl silica gel (found: C, 2.33%; H, 0.58%; N, 0.44%). Based on the elemental analysis of CSP **2**, the maximum loading of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** per gram of stationary phase is caculated to be 0.15 mmoles.
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