Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 18 (2007) 2437–2441

An expedient route for the practical preparation of optically active (−)-gossypol

Hai-Xia Jiang, Xing-Xin Cao, Hao Huang* and Biao Jiang*

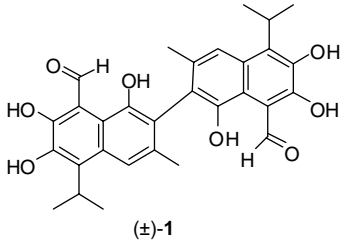
State Key Laboratory of Organometallic Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Road, Shanghai 200032, China

Received 9 August 2007; accepted 27 September 2007

Abstract—A simple and practical procedure has been developed for the resolution of racemic gossypol. The commercially available L-amino acid esters have been employed as the resolving agents with the L-tryptophan methyl ester (L-Trp-OMe) as the best reagent of choice. The individual diastereoisomeric gossypol adducts derived from L-Trp-OMe are readily separated by a simple filtration step to give the (−)-diastereoisomeric adduct, and its (+)-diastereoisomeric adduct can be easily obtained by simple evaporation of the mother liquor. Acid hydrolysis of the separated adduct gave (−)-gossypol and (+)-gossypol, respectively, in high chemical yields (quantitatively) and in high enantiomeric excesses (>95%).

1. Introduction

Gossypol[1,1′,6,6′,7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl-(2,2′-binaphthalene)-8,8′-dicarboxaldehyde] **1** (Fig. 1) is a natural bis-naphthalene product that is present in the members of the Malvaceae family, such as the cotton plant (*Gossypium* species) and the tropical tree *Thespesia populnea*,[1] which was discovered at the end of the 19th century by Longmore and Marchlewski;[2] its chemical structure was determined and confirmed by the total synthesis at 1935.[3] As a potentially valuable natural product with useful physiological and chemical properties to be exploited, gossypol **1** has been studied extensively since the discovery of its reversible male anti-fertility activity announced by Chinese scientists in the late 1970s.[4] However, the subsequent development of its clinical use as a male contraceptive was retarded owing to its serious side effects including hypokalemia (potassium shortage in the blood, which can lead to serious heart problems) and an irreversible anti-fertility effect.[5] More recently, new interest has developed in gossypol and significant attention has been focused on its potential therapeutic value as anti-tumor, antiproliferative, anti-apoptotic, anti-inflammatory, antimalarial, antioxidant, and antiproliferative agents.[6] Furthermore, gossypol **1** represents a promising starting point for the development of antitumor or antiviral derivatives for medicinal applications with enhanced bioactivity and reduced side effects.[7]



(±)-**1**

Figure 1. Chemical structure for racemic gossypol.

As a result of the restricted rotation about the 2,2′-binaphthyl bond, gossypol **1** exhibits atropisomerism, resulting in two optically active forms, L- or (−)-**1**, and D- or (+)-**1**. The (−)-enantiomer is usually more potent in all the biological systems tested, when compared to the (+)-enantiomer and the racemic mixture *rac*-**1**. It has been suggested that the (−)-gossypol in low concentrations affects cells in a stereospecific manner, whereas non-stereospecific interactions are found for (+)-gossypol and higher concentrations of (−)-gossypol.[8] The more recent finding of its binding to Bcl-2/Bcl-xL protein family afforded a plausible molecular

* Corresponding authors. Tel.: +86 21 54925470; fax: +86 21 64166128 (H.H.); e-mail addresses: huangh2003@mail.sioc.ac.cn; jiangb@mail.sioc.ac.cn

0957-4166/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2007.09.032

explanation of its capability to stop cancer, by triggering apoptosis.[9] The significance of this finding is that anti-apoptotic proteins Bcl-2 and Bcl-xL are overexpressed in many cancers, making them resistant to drug and radiation treatment. The latest discovery about the interaction of (−)-gossypol with another anti-apoptotic Bcl-2 member, Mcl-1 protein, indicates that (−)-gossypol represents a promising lead compound for the development of non-peptidic small-molecule inhibitors of Bcl-2, Bcl-xL, and Mcl-1.[10] As a result, (−)-gossypol may find its new applications in the fight against cancer and provide a scaffold for the synthesis of more promising pharmaceutics.[11] Toward this end, and to further our understanding of the mechanism by which (−)-gossypol exhibits its broad biological activities, more efficient methods for the practical preparation of enantiomeric (−)-gossypol in high chemical and enantiomeric purity are in high demand. To date, several approaches have been reported since early 1980s for the preparation of optically active gossypol,[12] including stereoselective synthesis and resolution methods.[13] The main drawbacks of the published methods were either too expensive or not amenable for practical preparation.[14] It is necessary to develop a practical method to produce (−)-gossypol in large quantities.

Herein, we report our convenient and efficient preparation of (−)-gossypol. To the best of our knowledge, our method provided the most practical route to produce (−)-gossypol in large quantities with both very good chemical and high enantiomeric purities. The process is simple to be carried out under mild condition and the products are easy to obtain by filtration and concentration. Our method provides the first example of the gram-scale preparation of optically active gossypol avoiding chromatographic methods, which is most suitable for more scaleable preparation.

2. Results and discussion

Racemic gossypol (±)-**1** was obtained from cotton seeds following our patented procedure.[15] Initially, we tried to resolve (±)-**1** using commercially available amino acids. Gossypol (±)-**1** was subjected to the treatment of chiral amino acids such as L-tryptophan (L-Trp-OH), L-tyrosine (L-Tyr-OH), and L-phenylalanine (L-Phe-OH), but the results were unsatisfactory as the reaction gave a mixture of water-soluble diastereomeric adducts, which were inseparable by recrystallization or flash chromatography. We next examined the reactions of gossypol (±)-**1** with a few amino acid esters including L-tryptophan methyl ester (L-Trp-OMe), L-tyrosine methyl ester (L-Tyr-OMe), and L-phenylalanine methyl ester (L-Phe-OMe) in several solvents including CH_2Cl_2, ethyl ether, and methanol, respectively. Finally methanol was identified as the ideal choice for the resolution. Thus, L-tryptophan methyl ester hydrochloride (L-Trp-OMe·HCl) was neutralized with NaOH in methanol and the racemic gossypol (±)-**1** was added into the stirring reaction mixture at 50 °C.

The progress of the reaction was monitored by TLC. The first promising result was observed with L-phenylalanine methyl ester (L-Phe-OMe), which gave a crystalline precipitate after the completion of the adducting reaction containing the (−)-diastereomeric adduct **2a** admixed with a small amount of the (+)-adduct **2b**. Better results were obtained with L-tyrosine methyl ester (L-Tyr-OMe), which afforded the corresponding (−)-diastereomeric adduct **3a** precipitating from the reaction solution, while (+)-**3b** and a small amount of (−)-**3a** remained in the mother liquor. Following this lead, our best results were obtained when L-tryptophan methyl ester (L-Trp-OMe) was tested as the resolving reagent. The success was achieved by treating gossypol (±)-**1** with L-Trp-OMe·HCl in MeOH in the presence of equimolar NaOH. The (−)-diastereomeric adduct **4a** {(*Rg*,*S*)-**4**, 95% de determined by ^{1}H NMR} {$[\alpha]_D^{19.7} = -1091$ (*c* 0.255, $CHCl_3$)} was found to crystallize out first from the reaction system in very good chemical yield (100%) in a single crystallization step. The (+)-diastereomeric adduct **4b** was also easily obtained by evaporation of the mother liquor and recrystallization in chloroform to give the highly pure **4b** {(*Sg*,*S*)-**4**, >99% de determined by ^{1}H NMR} {$[\alpha]_D^{26} = +288$ (*c* 0.55, CH_3OH)}. The characteristic lowerfield resonance of the NH [13.51 (br t, $J = 9.3$ Hz, –NH in **4a**) and 13.50 (br t, $J = 9.3$ Hz, –NH in **4b**)] verified the *Z*-configuration for the enamino group owing to the intramolecular hydrogen-bonding interaction between NH and C=O, which was consistent with the reported conclusion (Fig. 2).[14j]

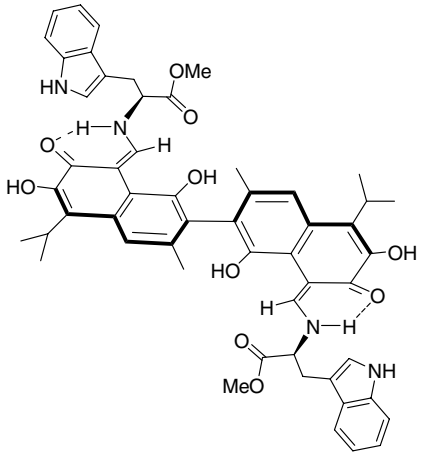
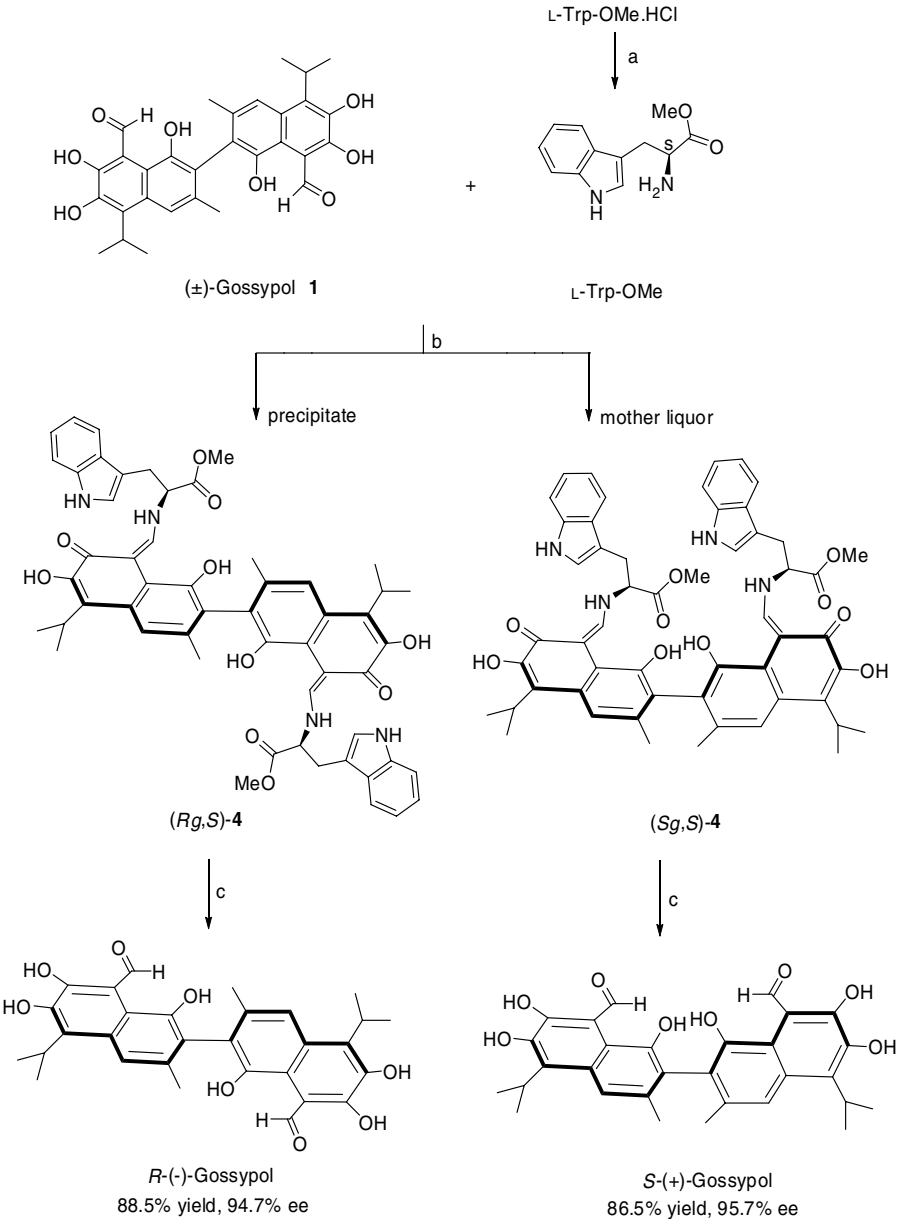


Figure 2. Chemical structure for (*Rg*,*S*)-**4**.

To liberate the resolved gossypol from the diastereomerically pure adducts **2a/b**–**4a/b**, the individual adduct was dissolved in ethyl ester and treated with acetic acid and hydrochloric acid. Filtration, extraction, and evaporation of the solvent directly afforded pure optically active (−)- and (+)-gossypols in quantitative yields, respectively (Scheme 1). The ^{1}H NMR spectra indicated greater than 98% purity with peaks at δ 11.13 (s, 2H, CHO), 7.78 (s, 2H, Ar-H), 6.43 (s, 2H, –OH), 5.87 (s, 2H, –OH), 3.89 (m, 2H, $CH(CH_3)_2$), 2.15 (s, 6H, Ar-CH_3), 1.55 (d, $J = 6.9$ Hz, 12H, $CH(CH_3)_2$). This is in excellent agreement with the published data for gossypol.[16] The enantiomeric excess of the obtained enantiomeric gossypol was >95% ee as determined by chiral HPLC analysis. Most of the L-amino acid ester hydrochloride (65–80%) can be recovered and recycled.



Scheme 1. Reagents and conditions: (a) NaOH, rt; (b) CH_3OH, 50 °C, 1–2 h; (c) H_3^+O.

3. Conclusion

In conclusion, we have developed a new practical method for the resolution of (±)-gossypol. The commercially available L-Trp-OMe was successfully used as the resolving agent of choice. The highly diastereomerically pure (−)- and (+)-adducts can be obtained in a single step simply through filtration and concentration. The optically purified (−)- and (+)-gossypols can be quantitatively regenerated by acid hydrolysis.

The preparation of enantiomerically pure gossypol via L-tryptophan methyl ester possesses a number of advantages. This procedure is easy to carry out; it has a short period for preparation, and affords both enantiomers in high enantiomeric excess. This method should also be economical since most of the L-amino acid ester hydrochloride (65–80%) can be recovered and recycled. For the first time, our procedure makes it possible to prepare the optically active gossypol without the need to use column chromatography on a gram-scale. The efficient resolution of gossypol utilizing L-tryptophan methyl ester hydrochloride as a resolving agent proved to be the most attractive scaleable approach for the preparation of enantiomerically pure gossypol.

4. Experimental

4.1. General

Optical rotations were recorded on Perkin–Elmer 341MC instrument. ^{1}H NMR and ^{13}C NMR spectra were recorded

on a Bruker AMX-300. The chemical shifts are expressed in ppm and coupling constants are given in Hz. Microanalyses were carried out on a Heraeus Rapid-CHNO instrument. Chiral HPLC analysis was performed on a Chiralpak AD-H analytical column using *i*-PrOH–hexane–TFA = 30:70:0.1 as an eluent (0.9 ml/min), detected at 254 nm. The amino acid ester hydrochlorides were purchased from GL Biochem (Shanghai) Ltd. Racemic gossypol was extracted following our own procedure.[15]

4.2. L-Trp-OMe-(−)-gossypol (*Rg*,*S*)-4 and L-Trp-OMe-(+)-gossypol (*Sg*,*S*)-4

A solution of L-tryptophan methyl ester hydrochloride (21.45 g, 0.084 mol) and NaOH (3.35 g, 0.084 mol) in methanol was stirred at room temperature and the racemic gossypol (21.74 g, 0.042 mol) was added. The mixture was then allowed to warm to 50–54 °C. The progress of the reaction was monitored by TLC. After 1.5–2 h, the reaction slurry was brought to room temperature and the yellow precipitate was filtered. This was then washed with methanol, and dried under reduced pressure to offer 20.5 g of L-Trp-OMe-(−)-gossypol ((*Rg*,*S*)-**4**) (95% de determined by ^{1}H NMR), the yield is 100%. $[\alpha]_D^{19.7} = -1091$ (*c* 0.255, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 13.51 (br t, $J = 9.3$ Hz, –NH), 9.09 (d, $J = 12$ Hz, 2H), 7.98 (br s, indole NH), 7.90 (br s, –OH), 7.56 (d, $J = 7.8$ Hz, 2H), 7.51 (s, 2H), 6.99 (t, $J = 7.2$ Hz, 2H), 6.91 (br s, 2H), 6.74–6.82 (m, 4H), 4.71 (s, –OH), 4.47 (m, 2H), 3.82 (s, 6H), 3.68–3.78 (m, 2H), 3.59 (dd, $J = 14.4$, 3.6 Hz, 2H), 3.23 (dd, $J = 14.4$, 9.6 Hz, 2H), 2.00 (s, 6H), 1.53 (d, $J = 6.9$ Hz, 6H), 1.52 (d, $J = 6.9$ Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 173.4, 170.7, 161.6, 148.4, 147.0, 136.3, 131.9, 128.9, 127.8, 126.5, 124.4, 122.0, 119.7, 118.1, 115.5, 114.2, 111.1, 108.8, 103.3, 62.2, 53.1, 30.7, 27.4, 20.3, 20.2, 19.9. Anal. Calcd for $C_{54}H_{54}O_{10}N_4 \cdot 1/2CH_3OH$: C, 70.01; H, 6.04; N, 5.99. Found: C, 70.02; H, 5.91; N, 5.85.

The methanol mother liquor was concentrated to dryness. The residue was extracted with dichloromethane by stirring the suspension, leaving behind the white insoluble inorganic salt. The combined dichloromethane extract was concentrated under reduced pressure to obtain 21 g of L-Trp-OMe-(+)-gossypol (*Sg*,*S*)-**4**, to give a yield of 99%. This was recrystallized carefully from chloroform to give a light yellow powder (>99% de determined by ^{1}H NMR). $[\alpha]_D^{26} = +288$ (*c* 0.55, CH_3OH). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 13.50 (br t, $J = 9.3$ Hz, –NH), 9.06 (d, $J = 11.7$ Hz, 2H), 8.08 (br s, indole NH), 7.98 (br s, –OH), 7.55 (s, 2H), 7.53 (d, $J = 7.8$ Hz, 2H), 7.20 (d, $J = 8.1$ Hz, 2H), 6.93–7.03 (m, 6H), 4.93 (br s, –OH), 4.42 (m, 2H), 3.75 (s, 6H), 3.68–3.75 (m, 2H), 3.52 (dd, $J = 14.4$, 4.2 Hz, 2H), 3.27 (dd, $J = 14.4$, 8.7 Hz, 2H), 2.03 (s, 6H), 1.53 (t, $J = 6.9$ Hz, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 173.3, 170.5, 161.7, 148.7, 147.0, 136.2, 131.7, 128.9, 127.6, 126.6, 124.2, 122.1, 119.7, 118.2, 117.9, 115.5, 114.4, 111.1, 108.8, 103.4, 62.7, 53.0, 30.3, 27.3, 20.3, 20.1. Anal. Calcd for $C_{54}H_{54}O_{10}N_4 \cdot CHCl_3$: C, 63.62; H, 5.34; N, 5.40. Found: C, 63.77; H, 5.36; N, 5.32.

4.3. (−)-Gossypol and (+)-gossypol

Acetic acid (0.9 ml) and a drop of hydrochloric acid (37%) were added in portions to a solution of (*Rg*,*S*)-**4** (92 mg, 0.1 mmol) in ether (6 ml). The mixture was heated at reflux for 1–1.5 h, with the progress of the reaction being monitored by TLC. After a while, a new white precipitate began to appear. The solid was washed with ether, dried, and established as L-tryptophan methyl ester hydrochloride (33 mg, 65%) to recycle. The ether mother liquor was treated with water, until the water phase showed neutrality (pH ≈ 7), and dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated to dryness under reduced pressure, and the residue was recrystallized carefully from Et_2O/PE (30–60 °C) to give 46 mg of a light yellow powdered (−)-gossypol. The yield is 88.5%, 94.7% ee by HPLC analysis [t_R 6.17 (major), t_S 23.8 (minor)]. $[\alpha]_D^{24.7} = -344.98$ (*c* 0.1, CH_3OH) {lit.[14j] $[\alpha]_D^{15} = -354.2$ (*c* 0.12, $CHCl_3$); $[\alpha]_D^{23} = -377$ (*c* 0.116, $CHCl_3$)}. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 11.13 (s, 2H, CHO), 7.78 (s, 2H, Ar-H), 6.43 (s, 2H, –OH), 5.87 (s, 2H, –OH), 3.89 (m, 2H, $CH(CH_3)_2$), 2.15 (s, 6H, Ar-CH_3), 1.55 (d, $J = 6.9$ Hz, 12H, $CH(CH_3)_2$).

(+)-Gossypol was obtained by the same method from L-Trp-OMe-(+)-gossypol (*Sg*,*S*)-**4** in 86.5% yield; 95.7% ee by HPLC analysis [t_R 6.14 (minor), t_S 24.2 (major)]. $[\alpha]_D^{26} = +364.9$ (*c* 0.22, CH_3OH) {lit.[14j] $[\alpha]_D^{30} = +357$ (*c* 0.10, $CHCl_3$); $[\alpha]_D^{15} = +375.6$ (*c* 0.115, $CHCl_3$)}. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 11.13 (s, 2H, CHO), 7.78 (s, 2H, Ar-H), 6.43 (s, 2H, –OH), 5.87 (s, 2H, –OH), 3.89 (m, 2H, $CH(CH_3)_2$), 2.15 (s, 6H, Ar-CH_3), 1.55 (d, $J = 6.9$ Hz, 12H, $CH(CH_3)_2$). And L-tryptophan methyl ester hydrochloride (40 mg, 78%) was recycled.

Acknowledgement

We thank the Shanghai Municipal Committee of Science and Technology for financial support.

References

1. Stipanovic, R. D.; Puckhaber, L. S.; Bell, A. A. *J. Agric. Food Chem.* **2006**, *54*, 1633–1637.
2. Wu, G. P.; Hou, J. Y. *Chin. J. Org. Chem.* **1985**, *4*, 193–204.
3. Kenar, J. A. *J. Am. Oil Chem. Soc.* **2006**, *83*, 269–302.
4. Anonymous *Chin. Med. J. (Engl.)* **1978**, *4*, 417–428.
5. Liang, X. S.; Rogers, A. J.; Webber, C. L.; Ormsby, T. J.; Tiritan, M. E.; Matlin, S. A.; Benz, C. C. *Invest. New Drugs* **1995**, *13*, 181–186.
6. Expert opinion on investigations on gossypol: past and present developments: Kalliopi, D. *Invest. Drugs* **2005**, *14*, 1419–1434.
7. See, for example: (a) Przybylski, P.; Bejcar, G.; Huczynski, A.; Schroeder, G.; Brzezinski, B.; Bartl, F. *Biopolymers* **2006**, *82*, 521–535; (b) Royer, R. E.; Mills, R. G.; Deck, L. M.; Mertz, G. J.; Jagt, D. L. V. *Pharmacol. Res.* **1991**, *24*, 407–412; (c) Lin, T. S.; Schinazi, R. F.; Zhu, J.; Birks, E.; Carbone, R.; Si, Y.; Wu, K.; Huang, L.; Pruwsoff, W. H. *Biochem. Pharmacol.* **1993**, *6*, 251–255.

8. Qiu, J.; Levin, L. K.; Buck, J.; Reidenberg, M. M. *Exp. Biol. Med.* **2002**, *227*, 398.
9. Kitada, S.; Marilisa, L.; Sareth, S.; Zhai, D. Y.; Reed, J. C.; Pellecchia, M. *J. Med. Chem.* **2003**, *46*, 4259–4264.
10. Wang, G.; Nikolovska-Coleska, Z.; Yang, C. Y.; Wang, R.; Tang, G.; Guo, J.; Shangary, S.; Qiu, S.; Gao, W.; Yang, D.; Meagher, J.; Stuckey, J.; Krajewski, K.; Jiang, S.; Roller, P. P.; Abaan, H. O.; Tomita, Y.; Wang, S. *J. Med. Chem.* **2006**, *49*, 6139–6142.
11. Tang, G.; Yang, C. Y.; Nikolovska-Coleska, Z.; Guo, J.; Qiu, S.; Wang, R.; Gao, W.; Wang, G.; Stuckey, J.; Krajewski, K.; Jiang, S.; Roller, P. P.; Wang, S. *J. Med. Chem.* **2007**, *50*, 1723–1726.
12. Michael, K. D. *Chirality* **2003**, *15*, 486–493.
13. Meyers, A. I.; Willemsen, J. J. *Chem. Commun.* **1997**, *16*, 1573–1574.
14. See, for example: (a) Matlin, S. A.; Zhou, R. *J. High Resolut. Chromatogr.* **1984**, *7*, 629–631; (b) Sampath, D. S.; Balaram, P. *J. Chem. Soc., Chem. Commun.* **1986**, *9*, 649–650; (c) Jpn. Kokai Tokkyo Koho JP01132542 A2 25 May 1989, Heisei, 3pp; (d) Zhao, A. N.; Wan, Y., et al. *Chin. J. Pharm. Anal.* **1991**, *11*, 1–3; (e) Yang, W. H.; Xiang, Sh. K. *Chin. J. Chromatogr.* **1995**, *13*, 264–266; (f) Wu, D. F.; Reidenberg, M. M.; Drayer Dennis, E. *J. Chromatogr.* **1988**, *433*, 141–148; (g) Matlin, S. A.; Belenguer, A., et al. *J. High Res. Chromatogr. Chromatogr. Commun.* **1987**, *10*, 86–91; (h) Matlin, S. A.; Zhou, R. H., et al. *Contraception* **1988**, *37*, 229–237; (i) Zheng, D. K.; Si, Y. K.; Meng, T. K.; Huang, L. *Acta Pharm. Sinica* **1990**, *25*, 417–422; (j) Zheng, D. K.; Si, Y. K.; Huang, L. *Acta Pharm. Sinica* **1990**, *25*, 423–434; (k) Zheng, D. K.; Huang, L.; Si, Y. K.; Meng, J. K. Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1033795A 12 Jul 1989, 35pp (People's Republic of China).
15. Huang H.; Cao X. X.; Jiang H. X. Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1887838A 3 Jan 2007, 6pp (People's Republic of China).
16. Brzezinski, B.; Olejnik, J.; Paszyc, S.; Aripov, T. F. *J. Mol. Struct.* **1990**, *220*, 261–268.