J|A|C|S ARTICLES

Published on Web 10/12/2010

A Unique Approach to the Concise Synthesis of Highly Optically Active Spirooxazolines and the Discovery of a More Potent Oxindole-Type Phytoalexin Analogue

Xianxing Jiang,[†,‡] Yiming Cao,[†] Yiqing Wang,[†] Luping Liu,[†] Fangfang Shen,[†] and Rui Wang*[,†,‡]

Key Laboratory of Preclinical Study for New Drugs of Gansu Province, Institute of Biochemistry and Molecular Biology, State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou, China, and State Key Laboratory of Chiroscience, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Kowloon, Hong Kong

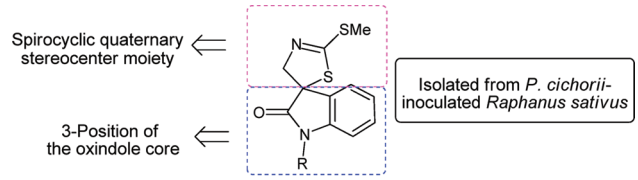
Received July 28, 2010; E-mail: wangrui@lzu.edu.cn

Abstract: Drug-lead synthesis through rapid construction of chiral molecular complexity around the biologically relevant framework using a highly efficient strategy is a key goal of organic synthesis. Molecules bearing a spirooxindole-type framework exhibit important bioactivities. Herein, we present a highly efficient and convenient strategy that allows rapid construction of unique optically active spiro[oxazoline-3,3′-oxindole]s through the organocatalyzed asymmetric synthesis of spirocyclic thiocarbamates via an aldol reaction. Preliminary biological evaluation of several of the spirooxazolines using a model of acute neuroinflammation revealed promising antipyretic activity and provided an opportunity to discover new antipyretic agents.

Introduction

The oxindole framework bearing a spirocyclic quaternary stereocenter at the C3 position represents a privileged heterocyclic motif commonly found in clinical pharmaceuticals and a number of natural spirooxindole alkaloids.[1] The oxindole-type phytoalexins[2] isolated from the plant family Cruciferae have shown potent antimicrobial, antitumor, and oviposition-stimulant biological activities (Scheme 1).[3] Since spiro[oxazoline-3,3′-oxindole] can be considered as an important analogue of the oxindole-type phytoalexins, the development of highly efficient synthetic methods to access optically active spirooxindole derivatives would be of great interest for drug-lead synthesis. Furthermore, it remains a great challenge to develop an efficient and convenient strategy to access these optically active natural alkaloids and related analogues for further biological studies, thereby contributing to the development of new therapeutic agents. On the basis of our recent efforts to build upon chiral precedents by using rosin-derived thiourea catalysts in our group[4,5] and to gain a better understanding of the scope of this conceptually new catalytic system, we hoped to expand our studies beyond model compounds to develop an efficient protocol for accessing potentially bioactive chiral spiro[oxazoline-3,3′-oxindole]s and to provide a foundation for further development of new types of therapeutic agents through preliminary biological studies.

Scheme 1. Structure of Oxindole-Type Phytoalexins



***Phytoalexin, Spirobrassinin*: R= H, *Methoxyspirobrassinin*: R= OMe**

Results and Discussion

We envisioned that the intramolecular aldol[6] cyclization reaction of α-isothiocyanato imides[7] to electron-deficient isatins could analogously be initiated in the presence of a chiral tertiary amine, leading to the generation of spiro[thiocarbamate-3,3′-oxindole]s. Subsequent methylation would afford spirooxazolines, which are isosteric analogues of natural spirobrassinin (Scheme 2). To our knowledge, there is no precedent for the catalytic asymmetric synthesis of highly optically active spiro[oxazoline-3,3′-oxindole]s, and the current protocol provides an alternative asymmetric access to chiral spirooxazolines. Herein, we first present our contribution to the successful development of such a reaction and preliminary biological studies of the activity toward the antipyretic role in the development of acute neuroinflammation.

To explore the possibility of the proposed aldol cyclization process, our investigation began with screening of several

[†] Lanzhou University.
[‡] The Hong Kong Polytechnic University.

(1) For recent reviews, see: (a) Trost, B. M.; Jiang, C. *Synthesis* **2006**, 369. (b) Williams, R. M.; Cox, R. J. *Acc. Chem. Res.* **2003**, *36*, 127. (c) Galliford, C. V.; Scheidt, K. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 8748. (d) Marti, C.; Carreira, E. M. *Eur. J. Org. Chem.* **2003**, 2209. (e) Lin, H.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2003**, *42*, 36.
(2) (a) Suchý, M.; Kutschy, P.; Monde, K.; Goto, H.; Harada, N.; Takasugi, M.; Dzurilla, M.; Balentová, E. *J. Org. Chem.* **2001**, *66*, 3940. (b) Takasugi, M.; Monde, K.; Katsui, N.; Shirata, A. *Chem. Lett.* **1987**, 1631.
(3) For selected examples, see: (a) Pedras, M. S. C.; Okanga, F. I.; Zaharia, I. L.; Khan, A. Q. *Phytochemistry* **2000**, *53*, 161. (b) Baur, E.; Stãdler, K.; Monde, K.; Takasugi, M. *Chemoecology* **1998**, *8*, 163. (c) Mehta, R. G.; Liu, J.; Constantinou, A.; Hawthorne, M.; Pezzuto, J. M.; Moon, R. C.; Moriarty, R. M. *Anticancer Res.* **1994**, *14*, 1209.

10.1021/ja106349m © **2010 American Chemical Society**

Scheme 2. Strategy for Synthesis of Chiral Spirooxazolines Using Chiral Tertiary Amines

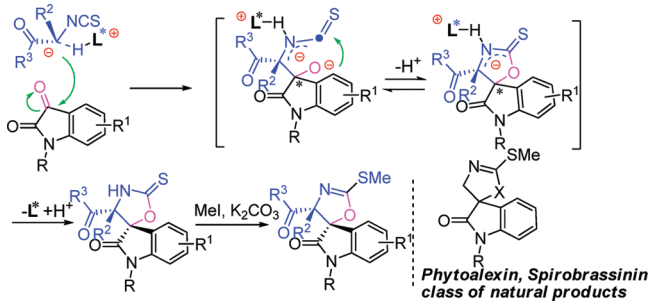
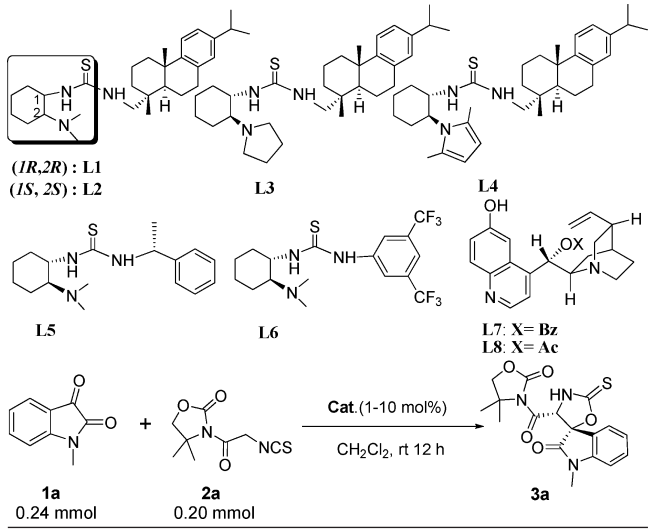


Table 1. Synthesis of Spiro[thiocarbamate-3,3′-oxindole] **3a** Using Various Organocatalysts and Optimization of the Reaction Conditions[a]

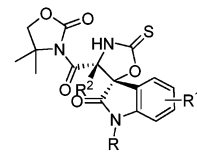
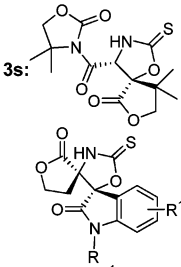


Entry	Cat.	Yield (%)[b]	dr[c]	ee (%)[d]
1	**TEA**	71	>99:1	0
2	**DIEA**	76	>99:1	0
3	**L1**	92	>99:1	80
4	**L2**	93	>99:1	91
5	**L3**	98	>99:1	96
6	**L4**	10	n.d	n.d.
7	**L5**	65	>99:1	62
8	**L6**	90	>99:1	86
9	**L7**	79	>99:1	76
10	**L8**	76	>99:1	75
11[e]	**L3**	98	>99:1	95
12[f]	**L3**	96	>99:1	96
13[g]	**L3**	36	>99:1	66

[a] Unless otherwise noted, the reaction was conducted with **1a** (0.24 mmol) and **2a** (0.20 mmol) in CH_2Cl_2 (1.0 mL) for 12 h at room temperature. [b] Isolated yield. [c] Determined by ^{1}H NMR spectroscopy. [d] The ee values were determined by HPLC, and the configuration was assigned by comparison with HPLC and X-ray crystal data for **3b**. [e] Using 5.0 mol % ligand loading. [f] Using 3.0 mol % ligand loading. [g] Using 1.0 mol % ligand loading.

organocatalysts to evaluate their catalytic activities. The model reaction of *N*-methylisatin (**1a**) with isothiocyanate **2a** was performed in CH_2Cl_2 at room temperature in the presence of a 10 mol % loading of ligand (Table 1). Besides the chiral tertiary amine−thiourea catalysts **L1**−**L6** with diversely structured scaffolds, the quinidine-derived bifunctional catalysts **L7** and **L8**[8] as well as the two organic bases triethylamine (TEA) and diisopropylethylamine (DIEA) were also tested. While the desired products could be obtained in moderate yields with excellent diastereoselectivities in the presence of TEA or DIEA,

Table 2. Spiro[thiocarbamate-3,3′-oxindole]s Formed by Rosin-Derived Tertiary Amine−Thiourea-Catalyzed Asymmetric Aldol Reaction[a]

Entry	*t* (h)	Product[b]	Yield (%)[c]	dr[d]	ee (%)[e]
					
1	12	**3a:** R= Me, R^1= H, R^2= H	96	>99:1	96%
2	12	**3b:** R= Me, R^1= 5-Br, R^2= H	81	>99:1	97%
3	12	**3c:** R= Me, R^1= 5-Cl, R^2= H	75	>99:1	>99%
4	12	**3d:** R= Me, R^1= 5-F, R^2= H	83	>99:1	>99%
5	12	**3e:** R= Me, R^1= 7-F, R^2= H	80	>99:1	98%
6	12	**3f:** R= Me, R^1= 5-OCF_3, R^2= H	91	>99:1	99%
7	12	**3g:** R= Me, R^1= 4,7-diCl, R^2= H	86	95:5	>99%
8	12	**3h:** R= Me, R^1= 5-Me, R^2= H	98	>99:1	97%
9	12	**3i:** R= Me, R^1= 5-OMe, R^2= H	99	>99:1	>99%
10	12	**3j:** R= Me, R^1= 5-Cl, 7-Me, R^2= H	89	>99:1	>99%
11	12	**3k:** R= Ph, R^1= H, R^2= H	94	86:14	93%
12	12	**3l:** R= Bn, R^1= H, R^2= H	90	>99:1	91%
13	12	**3m:** R= Allyl, R^1= H, R^2= H	99	95:5	95%
14[f]	96	**3n:** R= Me, R^1= H, R^2= Me	70	>99:1	>99%
15[f]	96	**3o:** R= Me, R^1= 5-Cl, R^2= Me	87	>99:1	99%
16[f]	96	**3p:** R= Me, R^1= 7-F, R^2= Me	80	>99:1	92%
17[f]	96	**3q:** R= Me, R^1= 5-F, R^2= Me	81	>99:1	>99%
18[f]	96	**3r:** R= Me, R^1= 5-Me, R^2= Me	79	>99:1	>99%
19	10	**3s:** 	99	>99:1	>99%
20	12	**3t:** R= Me, R^1= H	98	80:20	
21	12	**3u:** R= Bn, R^1= H	99	92:8	
22	12	**3v:** R= Ph, R^1= H	99	70:30	
23	12	**3w:** R= Allyl, R^1= H	92	83:17	

[a] For experimental details, see the Supporting Information. [b] The configuration was assigned by comparison with the HPLC and X-ray crystal data for **3b**. [c] Isolated yield. [d] Determined by ^{1}H NMR spectroscopy and chiral HPLC. [e] The ee values were determined by HPLC. [f] Using 10 mol % ligand loading.

no ee value was inevitably observed in the reactions (entries 1 and 2). Gratifyingly, except for **L4**, this catalytic asymmetric process generally exhibited efficacy in the presence of chiral organocatalysts. The results showed that tertiary amine−thiourea catalysts **L1**−**L3**, **L5**, and **L6** gave the products with the same excellent diastereoselectivities (>99:1 dr; entries 3−5, 7, and 8). Thiourea catalysts **L2** and **L3** provided excellent results in terms of yield and stereochemical outcome (entries 4 and 5), but **L3** furnished the products with slightly higher yield and enantioselectivity (98% yield and 96% ee; entry 5). In contrast, thiourea catalyst **L4** proved to be essentially inactive for this transformation (entry 6). Under the same reaction conditions, quinidine derivatives **L7** and **L8** showed relatively low catalytic activities in comparison with **L1**−**L3** and **L6**, affording the product **3a** in yields ranging from 79 to 76% and moderate enantioselectivities (76 and 75% ee, respectively; entries 9 and 10). The tertiary amine−thiourea catalyst **L3** was found to be the most promising catalyst for further investigation. In recent studies,[5b,c] we also tried to explain the role of structural features of thiourea catalysts in obtaining high enantioselectivity by

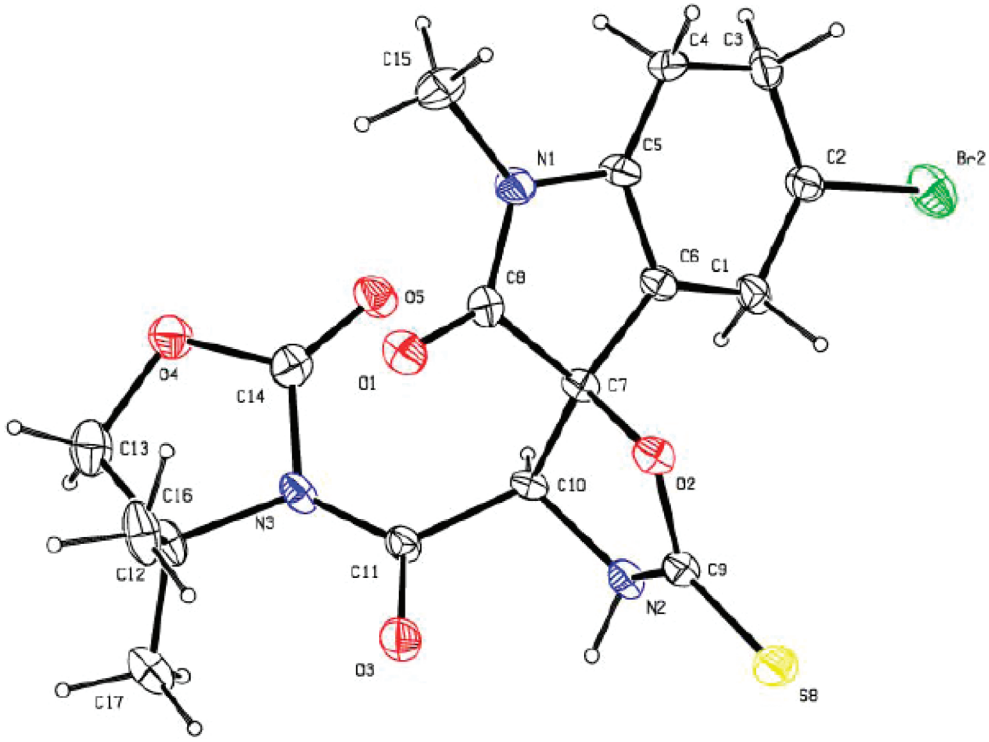


Figure 1. X-ray crystal structure of **3b**.

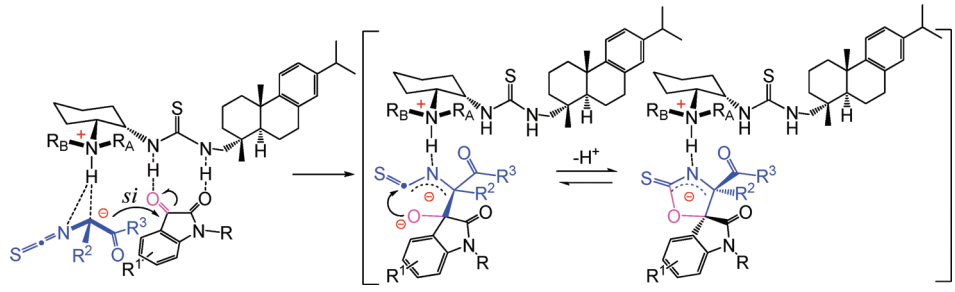


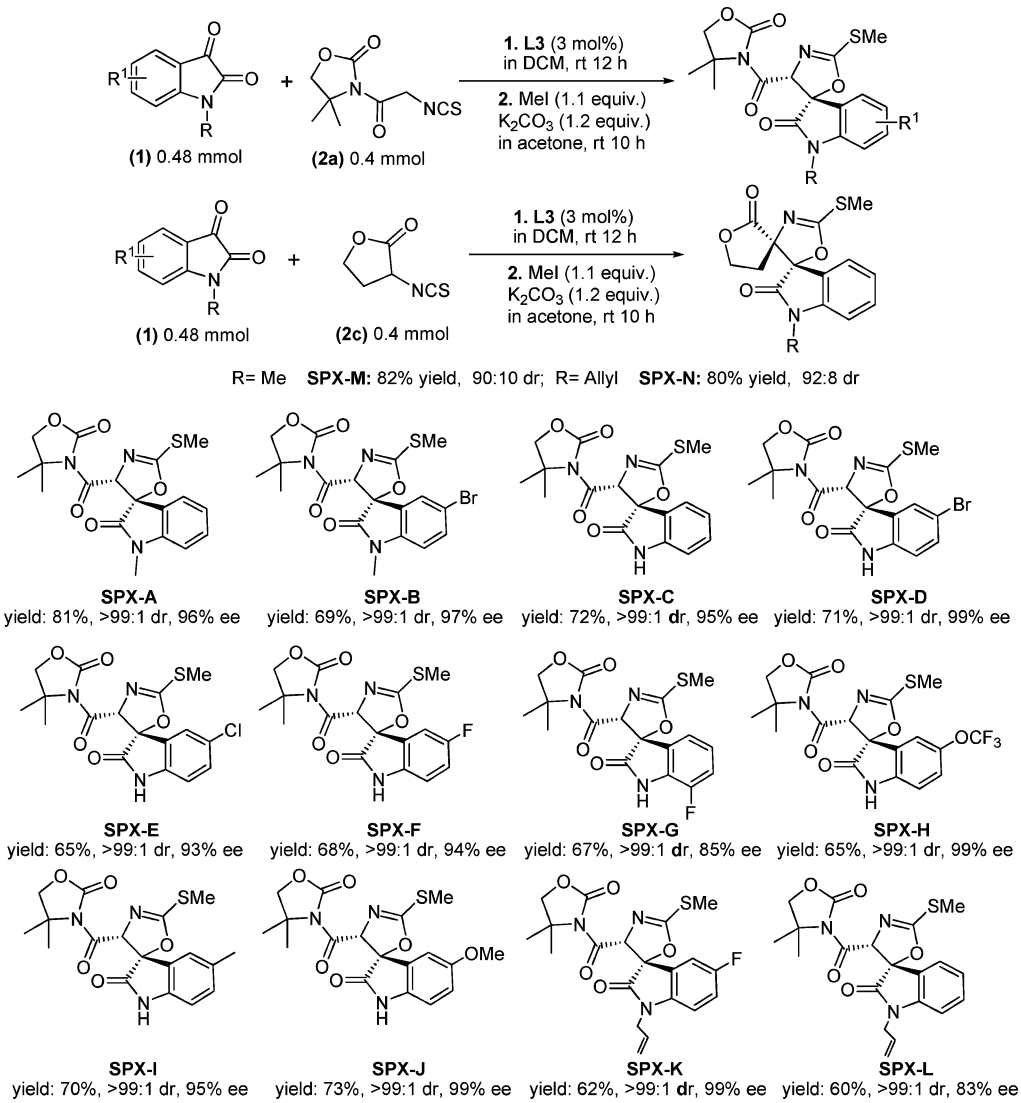
Figure 2. Proposed model of the reaction transition state.

carrying out reactions with catalysts bearing different configurations of the chiral scaffold moiety. The effects of the rosin-derived thiourea catalysts were investigated in comparison with other thiourea catalysts. We disclosed that the two chiral moieties of the thiourea are mutually reinforcing for the high efficacy of the catalyst. The stereochemical control of the reaction is mainly provided by the 1,2-diaminocyclohexane moiety of the thiourea. The catalytic activity depends mainly on the remaining chiral scaffold moiety of the thiourea (the inherent property of the stereochemical structure of the dehydroabietic amine moiety of the thiourea) and also on the suitable matching of the configuration of the 1,2-diaminocyclohexane moiety with the remaining chiral scaffold moiety. In addition, the excellent structural backbone and well-defined stereocenters of the dehydroabietic amine moiety of **L3** also have an important effect on the high enantioselectivity for the formation of adduct. These results also indicated that the excellent diastereoselectivity results from substrate stereocontrol in the reaction. To our delight, we further lowered the loading of catalyst **L3** to 3.0 mol % and still obtained 96% ee without a significant decrease in yield (96% yield; entry 12).

Results of experiments in which a variety of spirocyclic thiocarbamates were synthesized under the optimized conditions are summarized in Table 2. The results showed that variation of the electronic properties of the substituent at either C4, C5, or C7 of the N-protected spiro[thiocarbamate-3,3′-oxindole] with different steric parameters was tolerated, affording the products with excellent enantioselectivities (91%−99% ee) and diastereoselectivities in good to excellent yields (70%−99%; entries 1−18). To our delight, spirocyclic thiocarbamate **3s** was also favorably formed with 99% yield, >99:1 dr and >99% ee (entry 19). It is worth noting that the spiro[thiocarbamate-3,3′-oxindole]s bearing contiguous tetrasubstituted chiral carbon stereocenters[9] (**3n**−**r**) were still obtained in good yields and excellent stereoselectivities (entries 14−18). Additionally, as expected, the catalytic system also proved to be efficient for construction of contiguous dispiro[thiocarbamate-3,3′-oxindole]s, again leading to excellent yields (92−99%), albeit with a decrease in diastereoselectivity (entries 20−23). The relative and absolute configurations of the products were determined by X-ray crystal structure analysis of **3b** (see Figure 1).

On the basis of the experimental results described above and recent studies,[10] a possible model to account for the high enantioselectivity of the present reaction is shown in Figure 2. The rosin-derived chiral tertiary amine−thiourea is proposed as a bifunctional catalyst. The electron-deficient N-protected

Scheme 3. Synthesis of Biologically Active Chiral Spirooxazolines[a]



[a] Synthesis details are given in the Experimental Section.

isatin is activated by the two thiourea hydrogen atoms through weak hydrogen bonds, while the acidic α-carbon atom of the isothiocyanate could be activated by an interaction between the neighboring tertiary amine moiety of the catalyst and the isothiocyanate. The *si*-face of the N-protected isatin is predominantly approached by the incoming nucleophile generated from the isothiocyanate and the tertiary amine group of the bifunctional catalyst (attack on the *re*-face of the N-protected isatin is restricted by the cyclohexyl scaffold of the catalyst). Subsequent intramolecular cyclization reactions of intermediates afford the chiral spirocyclic products, which is consistent with the experimental results.

With the successful construction of spirocyclic thiocarbamates as described above, various optically active spirooxazolines were synthesized under the asymmetric protocol established next (Scheme 3; for details, see the Supporting Information). The substituted spiro[oxazoline-3,3′-oxindole]s **SPX-A** through **SPX-N** having various steric and electronic parameters, including in some cases *N*-methyl or *N*-allyl protection, were smoothly formed with good to excellent enantioselectivities (83% to 99% ee) and diastereoselectivities (up to >99:1 dr) in yields ranging from 60 to 82%.

The oxindole-type phytoalexin is widely known as an important component of the multifaceted defense systems of plants against microbial attack. Especially, in recent years, the interesting antimicrobial activity has been reported.[11] In view of the ease with which we were able to access diverse analogues

(4) For reviews concerning amine−thiourea catalysis, see: (a) Doyle, A. G.; Jacobsen, E. N. *Chem. Rev.* **2007**, *107*, 5713. (b) Connon, S. J. *Chem.—Eur. J.* **2006**, *12*, 5418. (c) Taylor, M. S.; Jacobsen, E. N. *Angew. Chem., Int. Ed.* **2006**, *45*, 1520. (d) Takemoto, Y. *Org. Biomol. Chem.* **2005**, *3*, 4299. For selected recent examples, see: (e) Tan, K. L.; Jacobsen, E. N. *Angew. Chem., Int. Ed.* **2007**, *46*, 1315. (f) Pan, S. C.; Zhou, J.; List, B. *Angew. Chem., Int. Ed.* **2007**, *46*, 612. (g) Yamaoka, Y.; Miyabe, H.; Takemoto, Y. *J. Am. Chem. Soc.* **2007**, *129*, 6686. (h) Zu, L. S.; Wang, J.; Li, H.; Xie, H. X.; Jiang, W.; Wang, W. *J. Am. Chem. Soc.* **2007**, *129*, 1036. (i) Wang, Y.; Li, H. M.; Wang, Y. Q.; Liu, Y.; Foxman, B. M.; Deng, L. *J. Am. Chem. Soc.* **2007**, *129*, 6364. (j) Wang, B. M.; Wu, F. H.; Wang, Y.; Liu, X. F.; Deng, L. *J. Am. Chem. Soc.* **2007**, *129*, 768. (k) Inokuma, T.; Hoashi, Y.; Takemoto, Y. *J. Am. Chem. Soc.* **2006**, *128*, 9413. (l) Huang, H.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2006**, *128*, 7170. (m) Wang, J.; Li, H.; Zu, L. S.; Jiang, W.; Xie, H. X.; Duan, W. H.; Wang, W. *J. Am. Chem. Soc.* **2006**, *128*, 12652. (n) Herrera, R. P.; Sgarzani, V.; Bernardi, L.; Ricci, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 6576. (o) McCooey, S. H.; Connon, S. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 6367. (p) Wang, J.; Li, H.; Duan, W. H.; Zu, L. S.; Wang, W. *Org. Lett.* **2005**, *7*, 4713.

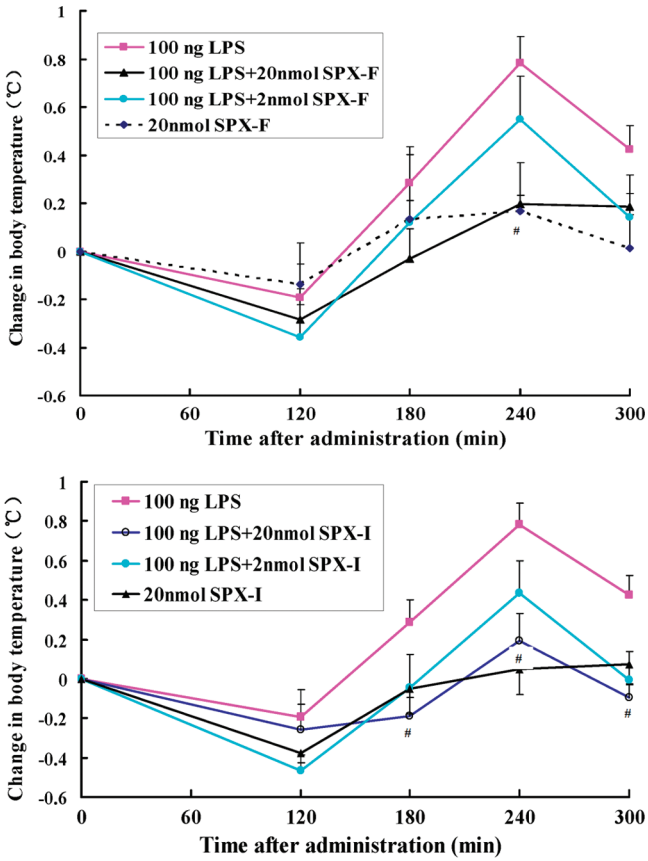


Figure 3. Biological activity of **SPX-F** and **SPX-I** in acute neuroinflammation. Time courses of the change in body temperature induced by LPS in the absence or presence of (top) **SPX-F** or (bottom) **SPX-I** injected into the third ventricle in mice are shown. The rectal temperature was recorded after injection of control, LPS, or the coapplication of LPS and **SPX-F** or **SPX-I**. Each data point represents the mean ± standard error of the mean from experiments conducted on 6−8 mice per group. Points labeled with # were significantly different ($p < 0.05$) from the corresponding points for LPS alone. (For experimental details, see the Supporting Information).

and the unique architecture of the optically active spiro[oxazoline-3,3′-oxindole]s formed, we decided to evaluate the biological activities of several spirooxazolines on fever by intracerebroventricular (icv) injection of lipopolysaccharide (LPS, a component of the outer membrane of Gram-negative bacteria) using a model of acute neuroinflammation in mice (Figure 3). The injection of LPS directly into the brain has been recognized as an animal model for the study of neuroinflammation.[12] Fever is part of the acute-phase reaction to infection, which is characterized by a raised thermoregulatory set point, leading to an elevation in body temperature.[13] Although fever is an important indicator for the severity of the inflammation, no one has investigated and/or linked this parameter with spirooxazolines during neuroinflammation. Using a model of acute neuroinflammation, we sought to determine the effects of the several spirooxazolines on the fever by icv injection of LPS. The current results demonstrate a role of the analogues in an animal model of acute neuroinflammation, pointing out their critical role in the fever induced by central administration of LPS. Excitingly, LPS-induced fever was significantly reduced by coinjection of several of the analogues, including **SPX-F** (20 nmol, $p < 0.05$) and **SPX-I** (20 nmol, $p < 0.05$); **SPX-F** (20 nmol) or **SPX-I** (20 nmol) given alone into the third ventricle did not significantly alter the body temperature. The two analogues administered at 2 nmol along with LPS slightly but not significantly reduced the LPS-induced fever. The realization that this kind of analogue plays a key role in the control of the process of neuroinflammation is a new concept and may well lead to a fruitful approach for identifying novel therapies for neuroinflammatory conditions. For example, it may be possible to use this kind of analogue as a therapeutic strategy to prevent and treat brain diseases associated with neuroinflammation (e.g., multiple sclerosis, Alzheimer's disease).

Conclusion

In summary, we have disclosed the synthesis of highly optically active spirooxazolines through organocatalyzed asymmetric synthesis of spirocyclic thiocarbamates with high levels of enantio- and diastereoselectivity (up to >99% ee and >99:1 dr) via an aldol reaction. Several of the new spirooxindoles were found to significantly reduce LPS-induced fever using a model of acute neuroinflammation. The preliminary biological studies on the activity toward the antipyretic role provide a foundation for further development of new spirooxindole-type antipyretic agents.

Experimental Section

General Procedure for the Synthesis of Highly Optically Active Spiro[oxazoline-3,3′-oxindole] (SPX-A). To a stirred solution of **L3** (0.012 mmol, 3.0 mol %) and *N*-methylisatin (**1a**) (0.48 mmol, 77.3 mg) in dry CH_2Cl_2 (2.5 mL) was added isothiocyanate **2a** (0.40 mmol, 85.6 mg) under Ar. The solution was stirred at room temperature for 12 h. After the reaction was complete (as determined by TLC), the resulting mixture was concentrated under reduced pressure, and the residue was purified through column chromatography on silica gel to give the optically pure spiro[thiocarbamate-3,3′-oxindole] **3a** (144.0 mg). To a mixture

(5) For rosin-derived thiourea, see: (a) Jiang, X. X.; Fu, D.; Zhang, G.; Cao, Y. M.; Liu, L. P.; Wang, R. *Chem. Commun.* **2010**, *46*, 4294. (b) Jiang, X. X.; Zhang, Y. F.; Chan, A. S. C.; Wang, R. *Org. Lett.* **2009**, *11*, 153. (c) Jiang, X. X.; Zhang, Y. F.; Liu, X.; Zhang, G.; Lai, L. H.; Wu, L. P.; Zhang, J. N.; Wang, R. *J. Org. Chem.* **2009**, *74*, 5562. (d) Jiang, X. X.; Zhang, Y. F.; Wu, L. P.; Zhang, G.; Liu, X.; Zhang, H. L.; Fu, D.; Wang, R. *Adv. Synth. Catal.* **2009**, *351*, 2096.

(6) (a) For a review of direct aldol reactions, see: *Modern Aldol Reactions*; Mahrwald, R., Ed.; Wiley-VCH: Weinheim, Germany, 2004. (b) For a review of organocatalytic aldol reactions, see: Mukherjee, S.; Yang, J. W.; Hoffmann, S.; List, B. *Chem. Rev.* **2007**, *107*, 5471.

(7) (a) Yoshino, T.; Morimoto, H.; Lu, G.; Matsunaga, S.; Shibasaki, M. *J. Am. Chem. Soc.* **2009**, *131*, 17082. (b) Li, L.; Ganesh, M.; Seidel, D. *J. Am. Chem. Soc.* **2009**, *131*, 11648. (c) Li, L.; Klauber, E. G.; Seidel, D. *J. Am. Chem. Soc.* **2008**, *130*, 12248. (d) Cutting, G. A.; Stainforth, N. E.; John, M. P.; Kociok-Köhn, G.; Willis, M. C. *J. Am. Chem. Soc.* **2007**, *129*, 10632. (e) Willis, M. C.; Cutting, G. A.; Piccio, V. J. D.; Durbin, M. J.; John, M. P. *Angew. Chem., Int. Ed.* **2005**, *44*, 1543.

(8) Li, H.; Wang, Y.; Tang, L.; Deng, L. *J. Am. Chem. Soc.* **2004**, *126*, 9906.

(9) For selected recent reviews, see: (a) Bella, M.; Gasperi, T. *Synthesis* **2009**, 1583. (b) Cozzi, P. G.; Hilgraf, R.; Zimmermann, N. *Eur. J. Org. Chem.* **2007**, 5969. (c) Douglas, C. J.; Overman, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5363.

(10) (a) Jiang, X. X.; Zhang, G.; Fu, D.; Cao, Y. M.; Shen, F. F.; Wang, R. *Org. Lett.* **2010**, *12*, 1544. (b) Zuend, S. J.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2007**, *129*, 15872.

(11) (a) Pedras, M. S. C.; Suchy, M.; Ahiahonu, P. W. K. *Org. Biomol. Chem* **2006**, *4*, 691. (b) Pedras, M. S. C.; Okanga, F. I.; Zaharia, I. L.; Khan, A. Q. *Phytochemistry* **2000**, *53*, 161. (c) Gross, D. *J. Plant. Dis. Prot.* **1993**, *100*, 433.

(12) Benamar, K.; Yondorf, M.; Barreto, V. T.; Geller, E. B.; Adler, M. W. *J. Pharmacol. Exp. Ther.* **2007**, *323*, 990.

(13) Benamar, K.; Yondorf, M.; Meissler, J. J.; Geller, E. B.; Tallarida, R. J.; Eisenstein, T. K.; Adler, M. W. *J. Pharmacol. Exp. Ther.* **2007**, *320*, 1127.

of **3a** and anhydrous K_2CO_3 (60.7 mg, 1.10 equiv) in 5.0 mL of acetone was added MeI (62.5 mg, 1.10 equiv) dropwise at 0 °C, after which the reaction was left overnight and the concentrated in vacuo. The mixture was subjected to chromatography to afford the purified product **SPX-A** (126.0 mg, 81% yield). The enantiomeric purity of the product was determined using HPLC, and the dr was determined using 300 Hz ^{1}H NMR spectroscopy. $[\alpha]_D^{20} = +115$ (c = 1.0, $CHCl_3$). Mp: 209 °C. Major diastereomer: ee = 96%, as determined by HPLC analysis (Chiralcel AD-H, *i*-PrOH/hexane = 30/70, 1.0 mL/min, 254 nm). Retention times: $t_{major} = 11.76$ min, $t_{minor} = 35.99$ min.

Materials and General Procedure for the Biological Studies. Male Kunming mice weighing 27−30 g were used. Each animal was used only once. The experiments were performed between 10:00 and 17:00. All mice were housed one per cage in a room maintained at 22 ± 0.5 °C and a relative humidity of 52 ± 2% with free access to food and water. The mice were placed in the specially designed restraining device as described by Rosow et al.[14] with their tails taped lightly to horizontal posts. Rectal temperatures were measured with a thermistor probe (Machine Equipment Corporation, GaoBeiDian, China) inserted to a depth of 2.5 cm into the rectum and linked to a recorder system (model BL-420E+, Taimeng Technology Corporation, Chengdu, China). The icv administration in the third ventricle was performed following the method described by Francés et al.[15] The drugs were coinjected to investigate whether the fever of LPS could be antagonized by the spiro[oxazoline-3,3′-oxindole]. Body temperature was recorded before and then at 120, 180, 240, and 300 min after icv injection in the third ventricle of control or various treatments. Changes in body temperature after injection and before drug administration were calculated for each animal. The time courses of the change in body temperature of mice subjected to different treatments are shown in Figure 3. Data were given as mean ± standard error of the mean. One-way ANOVA followed by Bonferroni's posthoc test was used to establish statistical significance; a probability level of $p < 0.05$ was considered to be significant.

Acknowledgment. This work is dedicated to Professor Albert S. C. Chan on the occasion of his 60th birthday. We are grateful for the grants from the NNSF of China (20932003 and 90813012) and the National S & T Major Project of China (2009ZX09503-017).

Supporting Information Available: Experimental details, compound characterization, and X-ray crystallographic data for **3b** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA106349M

(14) Rosow, C. E.; Miller, J. M.; Pelikan, E. W.; Cochin, J. *J. Pharmacol. Exp. Ther.* **1980**, *213*, 273.

(15) Frances, B.; Lahlou, H.; Zajac, J. M. *Regul. Pept.* **2001**, *98*, 13.