Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 8, pp. 867-872, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00131-X

SELECTIVE CYCLOOXYGENASE INHIBITORS: NOVEL 4-SPIRO 1,2-DIARYLCYCLOPENTENES ARE POTENT AND ORALLY ACTIVE COX-2 INHIBITORS

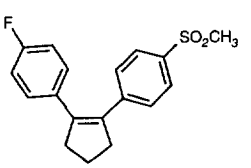
David B. Reitz*, Horng-Chih Huang, James J. Li, Danny J. Garland and Robert E. Manning
Medicinal Chemistry
Gary D. Anderson, Susan A. Gregory, Carol M. Koboldt, William E. Perkins,
Karen Seibert and Peter C. Isakson
Inflammatory Diseases Research

Searle Research & Development
c/o Monsanto Company
700 Chesterfield Parkway North
St. Louis, MO 63198

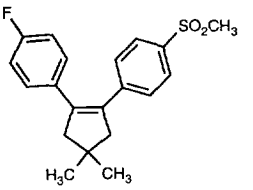
Abstract: Novel 4,5-diarylspiro[2.4]hept-5-enes, 6,7-diarylspiro[3.4]oct-6-enes, and 2,3-diarylspiro[4.4]non-2-enes have been synthesized and shown to be very potent inducible cyclooxygenase (COX-2) inhibitors with inhibition (IC_{50}) in the low nanomolar range and enzyme selectivity ratios as high as four orders of magnitude. The methyl sulfone spiro[2.4]hept-5-ene **1** (SC-58451) was found to be orally active (ED_{50} = 0.3 mpk) in the rat adjuvant-induced arthritis model with no gastric lesions observed at 200 mpk.

Non-steroidal antiinflammatory drugs (NSAIDs) are known to disrupt the production of prostaglandins by inhibiting the conversion of arachidonic acid to prostaglandins via constitutive cyclooxygenase (COX-1)[1,2] and ingestion of high doses of most common NSAIDs can produce side effects, including life-threatening ulcers, that limit their potential.[3] The recent discovery[4-6] of an inducible form of cyclooxygenase associated with inflammation has provided a novel target for therapeutic intervention. The selective inhibition of the inducible enzyme (COX-2) has the potential for more effective reduction of inflammation with fewer side effects.

Several laboratories have now reported examples of selective cyclooxygenase inhibitors which are orally active.[7-15] Recently, we have reported[16,17] that the 1,2-diarylcyclopentene methyl sulfone SC-57666[18] (COX-1



SC-57666



SC-58231

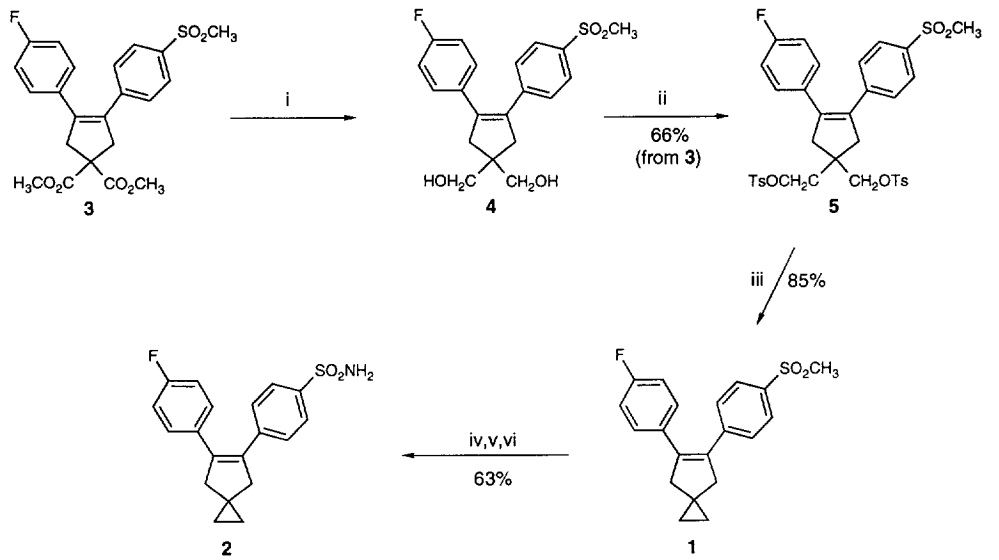
IC_{50} > 1000 μM[19], COX-2 IC_{50} = 0.026 μM) was orally active (ED_{50} = 1.7 mpk) in the rat adjuvant-induced arthritis model with no gastric or intestinal lesions at 200 mpk in rats and 600 mpk in mice. Substitution at the 4-position of 1,2-diaryl cyclopentenes with geminal alkyl groups produced analogs, e.g., the 4,4-dimethyl analog SC-58321 (COX-1 IC_{50} = 18.3 μM, COX-2 IC_{50} = 0.015 μM), which were generally less selective due to an increase in COX-1 activity.

As part of our continuing research efforts in the area of carbocyclic selective cyclooxygenase inhibitors, we have investigated 4-spiro analogs of SC-57666 in which the geminal methyl groups of SC-58231 have been joined together to form 3-, 4-, 5-, and 6-membered carbocyclic rings. We now report our results on methyl sulfone and sulfonamide analogs of 4,5-diarylspiro[2.4]hept-5-enes, 6,7-diarylspiro[3.4]oct-6-enes, 2,3-diarylspiro[4.4]non-2-enes, and 2,3-diarylspiro[4.5]dec-2-enes.

Chemistry

The synthesis of 5-(4-fluorophenyl)-6-[4-(methylsulfonyl)phenyl]spiro[2.4]hept-5-ene (**1**) and 4-[6-(4-fluorophenyl)spiro[2.4]hept-5-en-5-yl]benzenesulfonamide (**2**) from the 4,4-(dicarbomethoxy)cyclopentene methyl sulfone **3**[17] is outlined in Scheme 1. Reduction of a THF solution of **3** at -78 °C with diisobutylaluminum hydride (DIBAL-H) gave the 4,4-di(hydroxymethyl)cyclopentene **4**, which was subsequently treated with *p*-toluenesulfonyl chloride (TsCl) in pyridine to provide the corresponding ditosylate **5** in 66% overall yield. Treatment[20] of a DMF solurtion of **5** with NaI and zinc dust at 150 °C gave the methyl sulfone spiro[2.4]hept-5-ene inhibitor **1** in 85% yield. The corresponding sulfonamide inhibitor **2** was prepared in 63% yield from **1** using the recently reported methodology of Huang et al.[21]

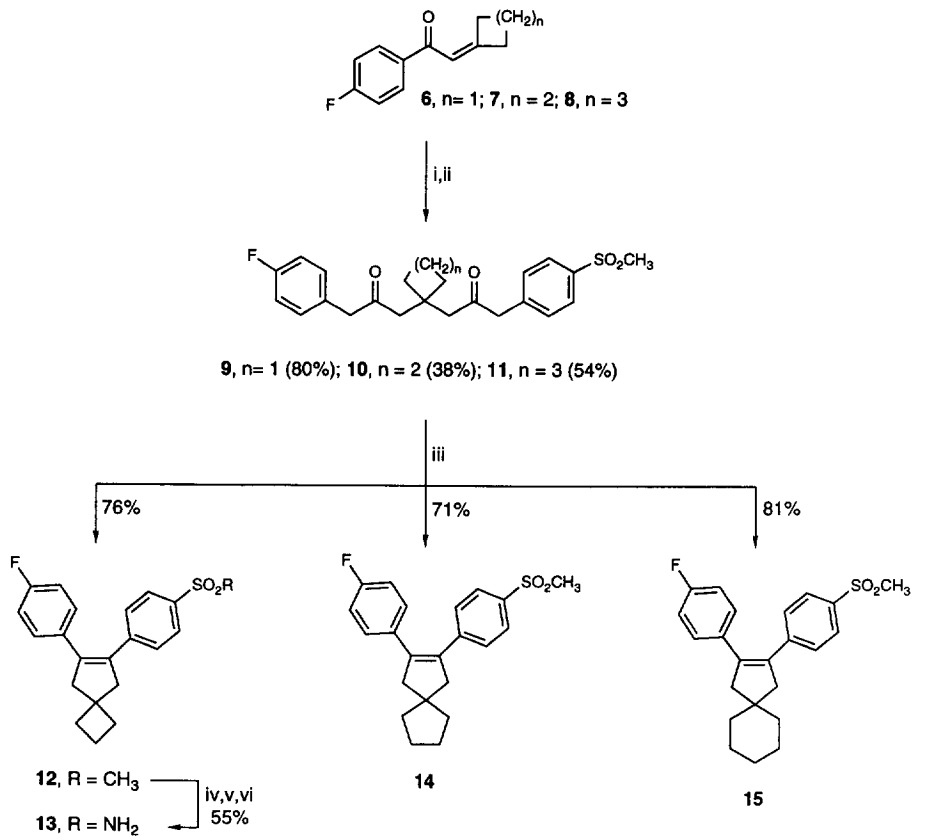
Scheme 1[a]



[a] Reagents: (i) DIBAL-H, THF, -78 °C; (ii) TsCl, pyridine; (iii) NaI, Zn^0, DMF, 150 °C; (iv) CH_3Li, THF, -78 °C; (iv) $(Bu)_3B$, Δ; (v) H_2NOSO_3H, NaOAc, H_2O.

The synthesis of 6-(4-fluorophenyl)-7-[4-(methylsulfonyl)phenyl]spiro3.4]oct-6-ene (**12**) and 4-[7-(4-fluorophenyl)spiro[3.4]oct-6-en-6-yl]benzenesulfonamide (**13**), 2-(4-fluorophenyl)-3-[4-(methylsulfonyl)-phenyl]spiro[4.4]non-2-ene (**14**), and 2-(4-fluorophenyl)-3-[4-(methylsulfonyl)-phenyl]spiro[4.5]dec-2-ene (**15**) from the α,β–unsaturated ketones **6**,[22-25] **7**,[22] and **8**,[26,27] respectively, is shown in Scheme 2. A methylene chloride solution of titanium(IV) chloride at -78 °C was treated with **6**, **7**, or **8** followed by the silyl enol ether of 4-(methylthio)acetophenone (prepared by reaction with chlorotrimethylsilane, sodium iodide, and triethylamine in acetonitrile)[23] to give the 1,5-diketones **9** in 80%, **10** in 38%, or **11** in 54%, respectively. McMurry coupling[28] of **9**, **10**, or **11** with titanium(iv) chloride and zinc metal in THF provided the methyl sulfones **12** in 76%, **14** in 71%, and **15** in 81%, respectively. Conversion of **12** to the spiro[3.4]oct-6-ene sulfonamide **13** in 55% was also accomplished via the Huang conversion.

Scheme 2[a]



[a] Reagents: (i) 4-$CH_3SC_6H_4C[OSi(CH_3)_3]=CH_2$,$TiCl_4$, CH_2Cl_2, -78 °C; (ii) MCPBA, CH_2Cl_2, 10 °C; (iii) $TiCl_4$, Zn^0,THF; (iv) CH_3Li, THF, -78 °C; (v) $(Bu)_3B$, Δ; (vi) H_2NOSO_3H, NaOAc, H_2O.

Results and Discussion

Table 1 presents the *in vitro* data for the inhibition (IC_{50}) of the constitutive (COX-1) and inducible (COX-2) forms of human recombinant cyclooxygenase[29] in the presence of 4-spiro 1,2-diarylcyclopentene inhibitors **1**, **2**, **12**, **13**, **14**, and **15**, together with comparative data for reference compounds NS-398[10,11] and indomethacin. Also shown in Table 1 is the *in vivo* data from the rat paw edema assay[30] which was used to assess the ability of orally administered (p.o.) compounds to inhibit inflammation caused by the introduction of carrageenan.

Table 1. Properties of 4-Spiro Cyclopentene Cyclooxygenase Inhibitors

compd[a]	COX-1[b] IC_{50} (μM)	COX-2[b] IC_{50} (μM)	selectivity[c]	Rat Paw Edema % inhibition[d]	log *P*
1	5.4	0.008	675	38	4.5
2	0.33	0.003	110	60	4.0
12	>100	0.004	>25,000	23	4.9
13	0.83	0.002	415	37	4.1
14	>100	0.062	>1600	10	5.4
15	>100	>100		e	e
NS-398	>100	0.1	>1000	60[f]	e
indomethacin	0.1	0.9	0.1	66[f]	e

[a] See ref 34. [b] See ref 29. [c] COX-1/COX-2. [d] Assay performed at 30 mpk (maximum response is 66%). [e] Not determined. [f] Assay performed at 10 mpk..

Connecting the geminal methyl groups of SC-58231 (COX-1 IC_{50} = 18.3 μM, COX-2 IC_{50} = 0.015 μM) to form a cyclopropyl ring, i.e., a spiro[2.4]hept-5-ene, produced the methyl sulfone **1** (COX-1 IC_{50} = 5.4 μM, COX-2 IC_{50} = 0.008 μM), an inhibitor which was about twice as potent and half as selective (1200 vs. 675) as SC-58231. The spiro[2.4]hept-5-ene sulfonamide **2** (COX-1 IC_{50} = 0.33 μM, COX-2 IC_{50} = 0.003 μM) was found to be even more potent and less selective (675 vs. 110) than the methyl sulfone **1**. Spiro[3.4]oct-6-enes, formed by connecting the geminal methyl groups of SC-58231 through a methylene spacer to form a cyclobutyl ring, proved to be very interesting inhibitors. The methyl sulfone spiro[2.4]oct-6-ene **12** (COX-1 IC_{50} >100 μM, COX-2 IC_{50} = 0.004 μM) was found to have slightly more COX-2 activity than the corresponding 3-membered ring analog **1**, while being essentially inactive on COX-1. Thus, a dramatic improvement in selectivity (>25,000 vs. 675) was observed for methyl sulfone analogs by expanding the size of the 4-spiro ring from cyclopropyl to cyclobutyl. The spiro[3.4]oct-6-ene sulfonamide **13** (COX-1 IC_{50} = 0.83 μM, COX-2 IC_{50} = 0.002 μM) was found to be the most potent COX-2 inhibitor of the series, although a concomitant increase in COX-1 activity of 2 orders og magnitude rendered **13** less selective than any of the methyl sulfones shown in Table 1. The methyl sulfone spiro[4.4]non-2-ene **14** (COX-1 IC_{50} >100 μM, COX-2 IC_{50} = 0.062 μM) was found to have substantially less COX-2 activity than either **1** or **12**. Thus, increasing the spiro-fused ring from cyclobutyl to cyclopentyl had a deleterious effect on COX-2 activity while having little (or no) effect on COX-1 activity. Finally, the methyl sulfone spiro[4.5]dec-5-ene **15** was found to be essentially inactive, thus supporting

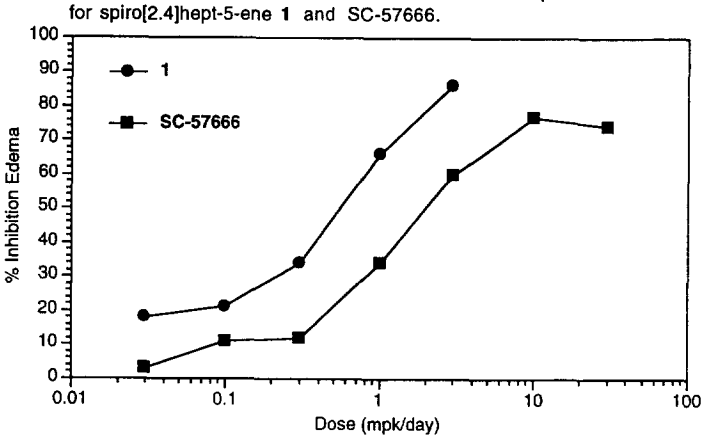
our previous inference[17] that the enzyme binding domain in this region is highly sensitive to inhibitor steric bulk.

The *in vivo* level of inhibition observed for the methyl sulfone spiro[2.4]hep-5-ene **1** (38% inhibition at 30 mpk) was found to be somewhat less than that reported for SC-58231 (50% inhibition at 30 mpk).[19] The sulfonamide **2** (60% inhibition at 30 mpk), however, was almost twice as active as **1**, and even slightly more active than SC-58231, in the rat paw edema assay. Both spiro[3.4]oct-6-enes **12** (23% inhibition at 30 mpk) and **13** (37% inhibition at 30 mpk) showed substantial decreases in *in vivo* activity relative to **1** and **2**, respectively, and the spiro[4.4]non-2-ene **14** was almost inactive in this assay. Log *P* values for 4-spiro 1,2-diarylcyclopentene cyclooxygenase inhibitors are listed in Table 1. An interesting correlation may be made between the log *P* values and the percent inhibition observed for 4-spiro cyclopentene cyclooxygenase inhibitors. The most potent analog (**2**) in the *in vivo* rat paw edema assay had the smallest log *P* value (4.0) and the least potent analog (**14**) had the largest log P value (5.4). Moreover, the consistent decrease in *in vivo* activity observed as log *P* values increased from 4.0 to 5.4 suggest that log *P* may have predictive value for this assay in this series. Additional *in vivo* studies were conducted to address GI toxicity.[31] No gastric lesions were observed in rats after 5 h when the methyl sulfone **1** (COX-1 IC_{50} = 5.4 μM) was administered intragastrically at 200 mpk, however, the sulfonamide **2** (COX-1 IC_{50} = 0.33 μM) showed gastric lesions in 10/10 rats at 200 mpk.

Figure 1 shows rat established adjuvant-induced arthritis[32] dose-response curves for the methyl sulfones spiro[2.4]hept-5-ene **1** (SC-58451) and for reference reference compound SC-57666. Spiro[2.4]hept-5-ene **1** (ED_{50} = 0.3 mpk) was found to have greater *in vivo* potency in this assay than the cyclopentene SC-57666 (ED_{50} = 1.7 mpk),[17] thus confirming that the addition of a 4-spirocyclopropyl ring is advantageous. Moreover, **1** is significantly more potent than NS-398 (ED_{30} = 4.7 mpk)[10] in the rat established adjuvant-induced arthritis model, even though NS-398 is more potent than **1** in the rat carrageenan-induced paw edema model (Table 1). It is not known currently whether the increased *in vivo* activity observed for **1** (relative to SC-57666) in rat adjuvant-induced arthritis is due largely to its greater COX-2 activity (IC_{50} = 0.026 μM vs. 0.008 μM), or is due to some combination of *in vivo* pharmacokinetic properties.

Studies with selective 4-spiro 1,2-diarylcyclopentene inhibitors are continuing. Spiro[2.4]hept-5-enes[33] are believed to have interesting biological properties and a more detailed report on them will appear elsewhere.

Figure 1. Rat Adjuvant-Induced Arthritis Dose-Response Curves for spiro[2.4]hept-5-ene **1** and SC-57666.



References and Notes

1. Vane, J. R. *Nature [New Biol.]* **1971**, *231*, 232-235.
2. Smith, J. B.; Willis, A. L. *Nature [New Biol.]* **1971**, *231*, 235-237.
3. Allison, M. C.; Howatson, A. G.; Torrance, C. J.; Lee, F. D.; Russell, R. I. G. *New Engl. J. Med.* **1992**, *327*, 749-754.
4. Hla, T.; Neilson K. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 7384-7388.
5. Xie, W.; Chipman, J. G.; Robertson, D. L.; Erikson, R. L.; Simmons, D. L. E. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 2692-2696.
6. Kujubu, D. A.; Fletcher, B. S.; Varnum, B. C.; Lim, R. W.; Herschman, H. R. T. *J. Biol. Chem.* **1991**, *266*, 12866-12872.
7. Gans, K. R.; Galbraith, W.; Roman, R. J.; Haber, S. B.; Kerr, J. S.; Schmidt, W. K.; Smith, C.; Hewes, W. E.; Ackerman, N. R. *J. Pharmacol. Exp. Ther.* **1990**, *254*, 180-187.
8. Copeland, R. S.; Williams, J. M.; Giannaras, J.; Nurnberg, S.; Covington, M.; Pinto, S.; Pick, S.; Trzaskos, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11202-11206.
9. Seibert, K.; Zhang, Y.; Leahy, S.; Hauser, S.; Masferrer, J.; Perkins, W.; Lee, L.; Isakson, P. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 12013-12017.
10. Futaki, N.; Yoshikawa, K.; Hamasaka, Y.; Arai, I.; Higuchi, S.; Iizuka, H.; Otomo, S. *Gen. Pharmac.* **1993**, *24*, 105-110.
11. Futaki, N.; Takahashi, S.; Yokoyama, M.; Arai, I.; Higuchi, S.; Otomo, S. *Prostaglandins* **1994**, *47*, 55-59.
12. Masferrer, J. L.; Zweifer, B. S.; Manning, P. T.; Hauser, S. D.; Leahy, K. M.; Smith, W. G.; Isakson, P.C.; Seibert, K. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 3228-3232.
13. Prasit, P. 208th National ACS Meeting, **1994**, MEDI-272.
14. Ford-Hutchinson, A. W.; Kennedy, B. P.; Prasit, P.; Vickers, P. J.; Lu, C-S.; Black, C..; Guay, D.; Lau, C. K.; Roy, P.; Eur. Pat. Appl. No. WO 94/13635 (6/23/94).
15. Isakson, P. 208th National ACS Meeting, **1994**, MEDI-270.
16. Reitz, D. B.; Li, J. J.; Norton, M.B.; Reinhard, E. J.; Penick, M. A.; Collins, J. T.; Logusch, E. W.; Garland, D. J.; Chamberlain, T. S.; Huang, H-C.; Isakson, P., Seibert, K.; Kobold, C.; Gregory, S.; Veenhuizen, A.; Zhang, Y.; Anderson, G.; Perkins, W.; Casler, J.; Ponte, C. 208th National ACS Meeting, **1994**, MEDI-271.
17. Reitz, D. B.; Li, J. J.; Norton, M. B.; Reinhard, E. J.; Collins, J. T.; Anderson, G. D.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Isakson, P. C. *J. Med. Chem.* **1994**, *37*, 3878-3881.
18. Reitz, D. B.; Li, J. U.S. Patent 5 344 991, 1994.
19. Reitz, D. B.; Li, J. J.; Norton, M. B.; Reinhard, E. J.; Huang, H-C.; Penick, M. A.; Collins, J. T.; Garland, D. J.; Seibert, K.; Koboldt, C.; Gregory, S. A.; Veenhuizen, A.; Zhang, Y.; Isakson, P. C. *Med. Chem. Res.* **1995**, *5*, in press.
20. Lerstrup, K.; Bailey, A.; McCullough, R.; Cowan, D.; Kistenmacher, T. *Synth. Metals*, **1987**, *19*, 647-652.
21. Huang, H-C.; Reinhard, E. J.; Reitz, D. B. *Tetrahedron Lett.* **1994**, *39*, 7201-7204.
22. The cyclobutyl unsaturated ketone **6** and cyclopentyl unsaturated ketone **7** were prepared in 65% and 11%, respectively, using procedures obtained from ref 23-25.
23. Walshe, N. D. A.; Goodwin, G. B. T.; Woodward, F. E.; *Org. Syn.* **1986**, *65*, 1-5.
24. Mukaiyama, T.; Narasaka, K. *Org. Syn.* **1986**, *65*, 6-11.
25. Narasaka, K. *Org. Syn.* **1986**, *65*, 12-15.
26. The cyclohexyl unsaturated ketone **8** was prepared in 36% using the procedure outlined in ref 27.
27. Lee, K.; Oh, D. Y. *Synthesis*, **1991**, 213-214.
28. McMurry, J. E. *Acc. Chem. Res.* **1983**, *16*, 405-411.
29. Gierse, J. K.; Hauser, S.D.; Creely, D. P.; Koboldt, C.; Rangwala, S. H.; Isakson, P.C.: Seibert, K. Biochem. J. **1995**, *305*, 479-484.
30. Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Soc. Exp. Bio. Med.* **1962**, *111*, 544-547.
31. Procedure used may be found in the Supplementary Material Section of ref 17.
32. Assay procedure used may be found in ref 7. Each data point represents an average of 8 animals with 95% confidence limits of ± 0.28 mpk.
33. Reitz, D. B.; Manning, R. E.; Huang, H-C.; Li, J. U.S. Patent 5 393 790, 1995.
34. All new compounds were fully characterized spectrally by ^{1}H NMR, MS, and HRMS; purity was established by a combination of analytical HPLC and combustion analysis (< ± 0.4%).

(*Received in USA* 1 *February* 1995; *accepted* 6 *March* 1995)