Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 5822–5826

Potent, selective pyrimidinetrione-based inhibitors of MMP-13

Lawrence A. Reiter,[a,*] Kevin D. Freeman-Cook,[a] Christopher S. Jones,[a] Gary J. Martinelli,[a] Amy S. Antipas,[a] Martin A. Berliner,[a] Kaushik Datta,[b] James T. Downs,[a] James D. Eskra,[a] Michael D. Forman,[a] Elaine M. Greer,[a] Roberto Guzman,[b] Joel R. Hardink,[a] Fouad Janat,[a] Nandell F. Keene,[a] Ellen R. Laird,[a] Jennifer L. Liras,[a] Lori L. Lopresti-Morrow,[a] Peter G. Mitchell,[a] Jayvardhan Pandit,[a] Donald Robertson,[b] Diana Sperger,[a] Marcie L. Vaughn-Bowser,[a] Darra M. Waller[a] and Sue A. Yocum[a]

[a]*Pfizer Global Research & Development, Groton Laboratories, Eastern Point Road, Groton, CT 06340, USA*
[b]*Pfizer Global Research & Development, Ann Arbor Laboratories, 2800 Plymouth Road, Ann Arbor, MI 48105, USA*

Received 18 July 2006; revised 11 August 2006; accepted 14 August 2006
Available online 30 August 2006

Abstract—Using SAR from two related series of pyrimidinetrione-based inhibitors, compounds with potent MMP-13 inhibition and >100-fold selectivity against other MMPs have been identified. Despite high molecular weights, c log *P*s, and polar surface areas, the compounds are generally well absorbed and have excellent pharmacokinetic (PK) properties when dosed as sodium salts. In a rat fibrosis model, a compound from the series displayed no fibrosis at exposures many fold greater than its MMP-13 IC_{50}.

We and others have reported the design of pyrimidinetrione-based inhibitors of matrix metalloproteases (MMP) and related proteases.[1–7] These proteases, having been implicated in an array of diseases and/or pathological states, have been the subject of intense research over the past two decades since inhibition of one or a small set of them may be sufficient to significantly alter the course of a specific disease or pathology. Our focus has primarily been on discovering disease modifying therapeutic agents for the treatment of osteoarthritis (OA).[8] In this regard, MMP-13 appears to be a prime target in that it is present in human OA cartilage tissue,[9,10] turns over type II collagen,[11] is co-localized with cleaved type II collagen in OA cartilage tissue,[12] and degrades cartilage.[13]

Perhaps the most significant impediment to the development of an MMP inhibitor for the treatment of any disease is musculoskeletal syndrome (MSS), a side effect clinically observed with non-selective MMP inhibitors. MSS is characterized by a stiffening of various joints and may derive from a lack of normal turnover of extracellular matrix due to inappropriate inhibition of one or more non-targeted MMPs.[14–17] The postulate that MMP-1 inhibition alone is responsible for the side effects was dispelled by clinical trials with CP-544439, a hydroxamic acid-based MMP-13 inhibitor that spared MMP-1 but which was otherwise non-selective.[18] This compound still produced MSS despite its lack of activity versus MMP-1. MMP-14 inhibition has also been proposed to cause MSS,[1] but this idea has not yet been clinically evaluated. In our most recent work we adopted a more conservative—but operationally more challenging—approach, seeking to identify a compound that potently inhibits MMP-13 while sparing other MMPs available to us (MMPs -1, -2, -3, -8, -9, -12, and -14). Of course, selectivity over this set did not guarantee universal selectivity for MMP-13. However, since the set contains representatives of both shallow- and deep-pocket S_1' proteases, we were confident that a compound selective against this set would have broad selectivity for MMP-13 over other MMPs.

Two observations made during the course of our investigation of the pyrimidinetrione series led us to believe

Keywords: Pyrimidinetrione; Matrix metalloprotease; MMP-13; Collagenase-3.
* Corresponding author. Tel.: +1 860 441 3681; e-mail: lawrence.a.reiter@pfizer.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.08.066

that the strategy posed above could be a success. First, as we recently reported,[1] a P_1' group containing a terminal heterocyclic group, and particularly an oxadiazole or oxazole, can lead to compounds with good MMP-13 potency and excellent selectivity (>100-fold) over MMPs -1 and -14. Second, in pursuing inhibitors containing P_1' terminal amides, we observed that reasonable levels of selectivity (>50-fold) over MMPs -2, -8, and -12 could be obtained with certain substitution patterns (see Table 1).[19,20] For example, the *N*-phenyl amide **1** is a potent MMP-13 inhibitor with 200-fold selectivity versus MMP-8 and the *N*-(3-fluorobenzyl) amide **4** displays 120-fold selectivity for MMP-13 versus MMP-12. The *N*-(3-pyridyl) amide **7** was among the most interesting of this set in that it displays >100-fold selectivity for MMP-13 versus both MMPs -8 and -12 and >50-fold selectivity over MMP-2.

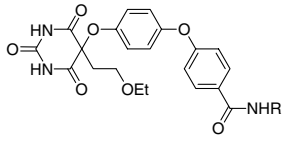
Although we were unable to identify an amide possessing the potency and selectivity attributes that we desired ($IC_{50} \leqslant 1$ nM with >100-fold potency over MMPs in our panel), the activity of compounds in the series suggested that compounds meeting our criteria might be found by combining structural features from the earlier reported heterocyclic and the amide series. The important features of the heterocyclic series were believed to be the position of the heteroatoms while in the amide series the overall length of the P_1' group and the presence and position of heteroatoms therein were deemed to be critical to the selectivity observed. The importance of the overall length of the P_1' group was reinforced by the X-ray crystal structure of **10** in MMP-13 (Fig. 1). This revealed significant space below the terminal fluorophenoxy group that could be occupied by a large, planar amide such as that in **7**. Furthermore, the selectivity observed in the amide series was likely due to the terminal residue contacting the loop at the base of the S_1' pocket, a region that is varied among the various MMPs with respect to the number and identity of the residues being present. This gave support to the premise that interactions with this region, both favorable and unfavorable, play an important role in determining selectivity. Accordingly, we sought to prepare heterocyclic variants of the *N*-aryl and/or *N*-benzyl amides and to this end, prepared seven different heterocyclic oxa-azole systems containing phenyl substituents on the heterocycles (see Table 2).[19,21]

Three of these systems, **15**–**17**, were not broadly pursued owing to their lower potency against MMP-13. Another, **14**, was not pursued owing to its relative lack of selectivity versus MMPs -2 and -12. The other series, represented by **11**–**13**, display good potency and promising selectivity. Two of these, the 4-aryloxazol-2-yl and 3-aryl-1,2,4-oxadiazol-5-yl systems (e.g., **12** and **13**, respectively), were investigated thoroughly with particular attention to the effects of substituents on the pendant aryl ring (see Table 3).[19,22]

In general the oxazoles are more potent than the oxadiazoles; however, one oxadiazole, the 4-pyridyl analog **23**, displayed potency and selectivity of the order that we sought. However, in vitro metabolism studies revealed this analog to have unacceptable turnover in microsomes and hepatocytes. In contrast, nearly all of the oxazoles displayed the potency sought (<1.0 nM); some, however, did not meet the selectivity criteria of being >100-fold more potent against MMP-13 versus the off-target MMPs. For example, the three pyridyl analogs **36**–**38** and the 3-cyano derivatives **32**–**33** did not meet the criterion with respect to MMP-2. Much of the SAR in the oxazole series was probed with both methoxyethyl and ethoxyethyl sides chains on the pyrimidinetrione. Although the potencies of the resulting pairs of analogs are generally very similar, the methoxyethyl analogs are somewhat more selective. This observation, plus the advantage that the methoxyethyl analogs have lower MW and clog *P*s, lead us to select two methoxyethyl analogs (**28** and **34**) for further studies.

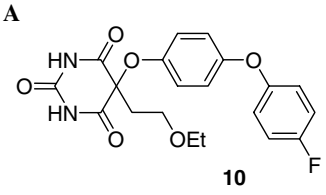
In our hamster model of MMP-13 inhibition,[23] a model that assesses the distribution of the compound into the knee joint and its ability to inhibit MMP-13 within the synovial fluid milieu, the 4-cyano derivative **34** was

Table 1. MMP IC_{50}s of P_1' terminal amides

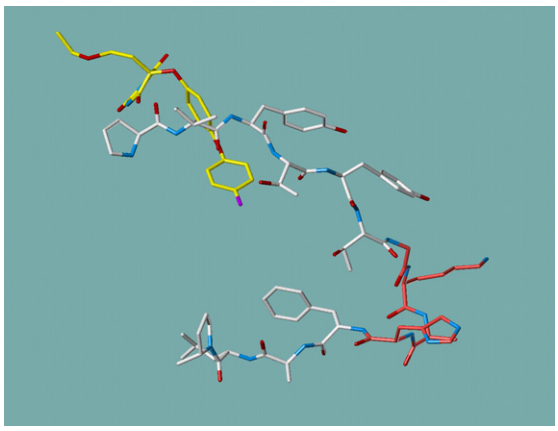


Compound	R	MMP-13	Fold selectivity		
		IC_{50} (nM)	MMP-2	MMP-8	MMP-12
1	–Ph	0.84	58	200	50
2	$–CH_2Ph$	8.0	21	ND	27
3	$–CH_2$-(2-F–Ph)	1.1	55	27	80
4	$–CH_2$-(3-F–Ph)	0.85	74	25	120
5	$–CH_2$-(4-F–Ph)	3.4	17	ND	11
6	–2-Pyridyl	3.9	27	15	27
7	–3-Pyridyl	0.40	63	120	190
8	$–CH_2$–(2-pyridyl)	6.1	17	ND	30
9	$–CH_2$–(3-pyridyl)	2.6	26	ND	27

A



10

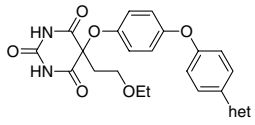


B

```
MMP-13: PIYTYTG-KSH-FMLP
MMP-8 : PNYAFRE-TSN-YSLP
MMP-2 : PIYTYTK-N---FRLS
MMP-12: PTYKYVD-I-NTFRLS
MMP-14: PFYQWMD-TEN-FVLP
MMP-9 : PMYRFTE---G-PPLH
MMP-3 : PLYHSLTDLTR-FRLS
MMP-1 : PSYTFSG---D-VQLA
```

Figure 1. (A) Compound **10** (yellow) bound in MMP-13 (variable region in red) (PDB: 1YOU). (B) Loop sequence alignment of MMPs (variable region in red).

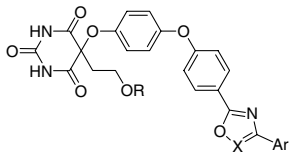
Table 2. MMP IC_{50}s of oxa-azole-based inhibitors



Compound	Heterocyclic system	MMP-13	Fold selectivity		
		IC_{50} (nM)	MMP-2	MMP-8	MMP-12
11		1.7	165	212	309
12		2.4	58	115	259
13		3.4	54	208	138
14		1.6	11	571	38
15		7.5	144	43	103
16		12	66	98	126
17		15	27	ND	37

found to give an ED_{50} of ~1 mg/kg while the 4-fluoro derivative **28** was about 10-fold less active. Compound **34** also displayed excellent pharmacokinetics (Clp 0.63 mL/min/kg, Vdss 0.34 L/kg, $t_{1/2}$ 6.9 h) in rats. Despite the compound having a MW > 500, a relatively high c log *P* of 3.9, and a polar surface area of 153, high absorption (80%) was observed when **34** was dosed as a spray dried dispersion. Compound **34** was also effective in preventing cartilage degradation (as measured by hydroxyproline release) from bovine nasal cartilage with

Table 3. MMP IC_{50}s of 3-Ar-1,2,4-oxadiazoles (X = N) and 4-aryloxazoles (X = CH)



Compound	R	X	Ar	MMP-13	Fold selectivity		
				IC_{50} (nM)	MMP-8	MMP-2	MMP-12
18	Et	N	2-F–Ph	3.3	167	102	176
19	Et	N	3-F–Ph	7.8	95	88	56
20	Et	N	4-F–Ph	6.1	210	80	114
21	Et	N	2-Pyridyl	1.0	271	61	71
22	Et	N	3-Pyridyl	2.0	320	134	504
23	Et	N	4-Pyridyl	0.54	555	291	232
24	Me	CH	2-F–Ph	1.4	149	288	605
25	Et	CH	2-F–Ph	0.93	270	562	868
26	Me	CH	3-F–Ph	0.46	152	239	1313
27	Et	CH	3-F–Ph	0.36	133	292	906
28	Me	CH	4-F–Ph	0.57	338	356	960
29	Et	CH	4-F–Ph	0.68	243	455	573
30	Me	CH	2-CN–Ph	0.73	516	173	342
31	Et	CH	2-CN–Ph	0.88	569	142	178
32	Me	CH	3-CN–Ph	0.63	140	29	181
33	Et	CH	3-CN–Ph	0.53	155	31	134
34	Me	CH	4-CN–Ph	0.36	394	397	619
35	Et	CH	4-CN–Ph	0.45	233	429	373
36	Et	CH	2-Pyridyl	1.0	57	74	158
37	Et	CH	3-Pyridyl	0.34	265	38	124
38	Et	CH	4-Pyridyl	0.40	250	70	530

an IC_{50} of 40 nM.[24] This potency level is somewhat better than that observed for the MMP-1-sparing, broad spectrum, hydroxamic acid-based MMP-13 inhibitor, CP-544439, which gave an IC_{50} of 70 nM in the same assay.[25]

With its excellent selectivity profile (as shown in Table 3 plus MMP-1: 2400-fold, MMP-3: 17,000-fold, MMP-9: 540-fold, and MMP-14: 540-fold) and pharmacokinetics much superior to the hydroxamic acid-based inhibitors that we previously examined,[18] **34** was well-suited to test the hypothesis that selective inhibition of MMP-13 would eliminate the MSS side effect seen with other MMP inhibitors. To this end, **34** was examined in the rat fibroplasia model, a surrogate for producing MSS in humans.[26] At doses of 30, 100, and 300 mg/kg bid for 14 days the compound was well tolerated and at the 100 mg/kg dose, a C_{max} of 198 μg/mL (~370 μM) and AUC of 3840 (μg h/mL) were obtained.[27] This C_{max} exceeds by >50-fold the rat MMP-13 IC_{50} of 7.2 μM (determined in rat plasma).[28] Even at the C_{min} of 129 μg/mL (~240 μM) the rat MMP-13 IC_{50} is exceeded >30-fold. Despite exposures well above the rat MMP-13 IC_{50}, no fibroplasia was observed.

In summary, we have described the discovery of a series of selective MMP-13 inhibitors. Selectivity was obtained by optimizing the P_1' heterocycle and its terminal substituent, both of which lie deep in the S_1' pocket. Generally, the ionizability of the pyrimidinetrione moiety confers good solubility to molecules in the series and good absorption can be obtained despite moderate to high MWs, clog *P*s, and polar surface areas. Molecules such as **34** should allow a conclusive assessment of whether an MMP-13 inhibitor can escape the spectre of MSS and provide protection to type II collagen in articular cartilage and relief to OA patients.

References and notes

1. Blagg, J. A.; Noe, M. C.; Wolf-Gouveia, L. A.; Reiter, L. A.; Laird, E. R.; Chang, S.-P. P.; Danley, D. E.; Downs, J. T.; Elliott, N. C.; Eskra, J. D.; Griffiths, R. J.; Hardink, J. R.; Haugeto, A. I.; Jones, C. S.; Liras, J. L.; Lopresti-Morrow, L. L.; Mitchell, P. G.; Pandit, J.; Robinson, R. P.; Subramanyam, C.; Vaughn-Bowser, M. L.; Yocum, S. A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1807.
2. Grams, F.; Brandstetter, H.; D'Alò, S.; Geppert, D.; Krell, H.-W.; Leinert, H.; Livi, V.; Menta, E.; Oliva, A.; Zimmermann, G. *Biol. Chem.* **2001**, *382*, 1277.
3. Foley, L. H.; Palermo, R.; Dunten, P.; Wang, P. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 969.
4. Dunten, P.; Kammlott, U.; Crowther, R.; Levin, W.; Foley, L. H.; Wang, P.; Palermo, R. *Protein Sci.* **2001**, *10*, 923.
5. Brandstetter, H.; Grams, F.; Glitz, D.; Lang, A.; Huber, R.; Bode, W.; Krell, H.-W.; Engh, R. A. *J. Biol. Chem.* **2001**, *276*, 17405.
6. Kim, S.-H.; Pudzianowski, A. T.; Leavitt, K. J.; Barbosa, J.; McDonnell, P. A.; Metzler, W. J.; Rankin, B. M.; Liu, R.; Vaccaro, W.; Pitts, W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1101.
7. Duan, J. J.-W.; Lu, Z.; Wasserman, Z. R.; Liu, R.-Q.; Covington, M. B.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2970.

8. Tardif, G.; Reboul, P.; Pelletier, J.-P.; Martel-Pelletier, J. *Mod. Rheumatol.* **2004**, *14*, 197.
9. Mitchell, P. G.; Magna, H. A.; Reeves, L. M.; Lopresti-Morrow, L. L.; Yocum, S. A.; Rosner, P. J.; Geoghegan, K. F.; Hambor, J. E. *J. Clin. Invest.* **1996**, *97*, 761.
10. Yocum, S. A.; Lopresti-Morrow, L. L.; Reeves, L. M.; Mitchell, P. G. *Ann. NY Acad. Sci.* **1999**, *878*, 583; Borden, P.; Solymar, D.; Sucharczuk, A.; Lindman, B.; Cannon, P.; Heller, R. A. *J. Biol. Chem.* **1996**, *271*, 23577.
11. Knauper, V.; Lopez-Otin, C.; Smith, B.; Knight, G.; Murphy, G. *J. Biol. Chem.* **1996**, *271*, 1544.
12. Freemont, A. J.; Byers, R. J.; Taiwo, Y. O.; Hoyland, J. A. *Ann. Rheum. Dis.* **1999**, *58*, 357.
13. Billinghurst, R. C.; Dahlberg, L.; Ionescu, M.; Reiner, A.; Bourne, R.; Rorabeck, C.; Mitchell, P.; Hambor, J.; Diekmann, O.; Tschesche, H.; Chen, J.; Van Wart, H.; Poole, A. R. *J. Clin. Invest.* **1997**, *99*, 1534; Billinghurst, R. C.; Wu, W.; Ionescu, M.; Reiner, A.; Dahlberg, L.; Chen, J.; Van Wart, H.; Poole, A. R. *Arthritis Rheum.* **2000**, *43*, 664.
14. Hutchinson, J. W.; Tierney, G. M.; Parsons, S. L.; Davis, T. R. C. *J. Bone Joint Surg. (Br)* **1998**, *80-B*, 907.
15. Wojtowicz-Praga, S.; Torri, J.; Johnson, M.; Steen, V.; Marshall, J.; Ness, E.; Dickson, R.; Sale, M.; Rasmussen, H. S.; Chiodo, T. A.; Hawkins, M. J. *J. Clin. Oncol.* **1998**, *16*, 2150.
16. Griffioen, A. W. *IDrugs* **2000**, *3*, 336.
17. Levitt, N. C.; Eskens, F. A. L. M.; O'Byrne, K. J.; Propper, D. J.; Denis, L. J.; Owen, S. J.; Choi, L.; Foekens, J. A.; Wilner, S.; Wood, J. M.; Nakajima, M.; Talbot, D. C.; Steward, W. P.; Harris, A. L.; Verweij, J. *Clin. Cancer Res.* **2001**, *7*, 1912.
18. Reiter, L. A.; Robinson, R. P.; McClure, K. F.; Jones, C. S.; Reese, M. R.; Mitchell, P. G.; Otterness, I. G.; Bliven, M. L.; Liras, J.; Cortina, S. R.; Donahue, K. M.; Eskra, J. D.; Griffiths, R. J.; Lame, M. E.; Lopez-Anaya, A.; Martinelli, G. J.; McGahee, S. M.; Yocum, S. A.; Lopresti-Morrow, L. L.; Tobiassen, L. M.; Vaughn-Bowser, M. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3389.
19. The pyrimdinetriones were prepared from the requisite 1-substituted-4-(4-hydroxyphenoxy)benzenes and either 5-bromo-5-ethoxyethyl- or 5-bromo-5-methoxyethylpyrimidinetrione as previously described (Ref. 1).
20. The 4-(4-hydroxyphenoxy)benzamides utilized to prepare **1**–**5** and **8**–**9** were prepared through a BOP-mediated coupling of the requisite amine with 4-(4-hydroxyphenoxy)benzoic acid. The 4-(4-hydroxyphenoxy)benzamides utilized to prepare **6**–**7** were prepared through a coupling of 4-(4-benzyloxyphenoxy)benzoyl chloride with the requisite pyridine amine followed by hydrogenolytic cleavage of the benzyl ether.
21. The 1-substituted-4-(4-hydroxyphenoxy)benzenes utilized to prepare **11**–**17** were prepared by: (**11**) a Suzuki reaction of 5-bromo-2-phenyloxazole and 4-(4-methoxyphenoxy)benzeneboronic acid followed by demethylation using methionine in neat methanesulfonic acid (Ref. 29); (**12**) a Negishi reaction of 4-phenyloxazole and 1-iodo-4-(4-methoxyphenoxy)benzene followed by demethylation. The iodide was prepared by iodination of 4-(4-methoxyphenoxy)benzene (Ref. 30); (**13**) a TBTU-mediated condensation of 4-(4-hydroxyphenoxy)benzoic acid with *N*-hydroxybenzenecarboxamidine (Ref. 31); (**14**) a Negishi reaction of 3-phenyl-5-(trifluoromethanesulfonyl)oxyisoxazole and 4-(4-methoxyphenoxy)benzeneboronic acid followed by demethylation; (**15**) a Negishi reaction of 5-phenyloxazole and 1-iodo-4-(4-methoxyphenoxy)benzene followed by demethylation; (**16**) a condensation of ethyl 4-(4-methoxyphenoxy)benzoate with hydrazine followed by acylation with benzoyl chloride, thionyl chloride-mediated ring closure, and demethylation; (**17**) a reaction of 4-(4-hydroxyphenoxy)benzenecarbonitrile with hydroxylamine followed by TBTU-mediated condensation with benzoic acid and demethylation.
22. The 1-substituted-4-(4-hydroxyphenoxy)benzenes utilized to prepare **18**–**38** were prepared: (**18**–**23**) analogously to **13** using the requisite aryl carboxylic acid; (**24**–**38**) by either a Suzuki or Negishi reaction of the requisite boronic acid (ester) or organozinc derivative and 2-[4-(4-methoxyphenoxy)phenyl]-4-(trifluoromethanesulfonyl)oxyoxazole followed by demethylation. The oxazole triflate was prepared according to the method of Kelly and Lang (Ref. 32).
23. Otterness, I. G.; Bliven, M. L.; Eskra, J. D.; te Koppele, J. M.; Stukenbrok, H. A.; Milici, A. J. *Osteoarthritis Cartilage* **2000**, *8*, 366.
24. Milner, J. M.; Elliot, S.-F.; Cawston, T. E. *Arthritis Rheum.* **2001**, *44*, 2084.
25. CP-544439: compound **10** in Ref. 18.
26. Renkiewicz, R.; Qiu, L.; Lesch, C.; Sun, X.; Devalaraja, R.; Cody, T.; Kaldjian, E.; Welgus, H.; Baragi, V. *Arthritis Rheum.* **2003**, *48*, 1742.
27. Drug exposures of animals at the 300 mg/kg dose were somewhat lower than the exposures at the 100 mg/kg dose.
28. The IC_{50} for **34** against rat MMP-13 is 4.7 nM. The compound is highly protein bound (>99%) which leads to a large shift in potency in rat plasma.
29. Andre, J. D.; Dormoy, J. R.; Heymes, A. *Synth. Commun.* **1992**, *22*, 2313.
30. Brewster, R. Q.; Choguill, H. S. *J. Am. Chem. Soc.* **1939**, *61*, 2702.
31. Poulain, R. F.; Tartar, A. L.; Deprez, B. P. *Tetrahedron Lett.* **2001**, *42*, 1495.
32. Kelly, T. R.; Lang, F. *J. Org. Chem.* **1996**, *61*, 4623.