# Metabolism-Based Identification of a Potent Thrombin Receptor Antagonist

Martin C. Clasby,*[,†] Samuel Chackalamannil,[†] Michael Czarniecki,[†] Dario Doller,[†] Keith Eagen,[†] William Greenlee,[†] Grace Kao,[†] Yan Lin,[†] Hsingan Tsai,[†] Yan Xia,[†] Ho-Sam Ahn,[‡] Jacqueline Agans-Fantuzzi,[‡] George Boykow,[‡] Madhu Chintala,[‡] Carolyn Foster,[‡] April Smith-Torhan,[‡] Kevin Alton,[§] Matthew Bryant,[§] Yunsheng Hsieh,[§] Janice Lau,[§] and Jairam Palamanda[§]

*Central Nervous System and Cardiovascular Chemical Research, Cardiovascular and Metabolic Disease Research, and Drug Metabolism and Pharmacokinetics Research, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033*

*Received August 30, 2006*

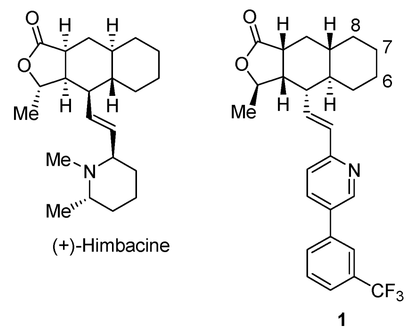
The metabolism of our prototypical thrombin receptor antagonist **1**, $K_i$ = 2.7 nM, was studied and three major metabolites (**2**, **4**, and **5**) were found. The structures of the metabolites were verified independently by synthesis. Compound **4** was shown to be a potent antagonist of the thrombin receptor with a $K_i$ = 11 nM. Additionally, compound **4** showed a 3-fold improvement in potency with respect to **1** in an agonist-induced ex-vivo platelet aggregation assay in cynomolgus monkeys after oral administration; this activity was sustained with 60% inhibition observed at 24 h post-dose. Compound **4** was highly active in functional assays and showed excellent oral bioavailability in rats and monkeys. Compound **4** showed a superior rat enzyme induction profile relative to compound **1**, allowing it to replace compound **1** as a development candidate.

## Introduction

Thrombin is a multifunctional protease involved in hemostasis and wound healing.[1] Thrombin plays a key role in the coagulation cascade by converting fibrinogen to fibrin which is then cross-linked to form a clot by trapping aggregated platelets, red blood cells, and other plasma particles;[2] the clot is then stabilized by factor XIIIa (fibrin-stabilizing factor), which itself is activated by thrombin. Additionally, thrombin promotes clot formation by upregulating its own production through activation of blood Factors V, VIII, and XI. In addition to its important roles in the coagulation cascade, thrombin activates a number of cell types such as platelets, leukocytes, endothelial cells, and vascular smooth muscle cells.[3,4] Thrombin is the most potent activator of platelets which normally has a beneficial effect on essential clot formation under normal physiological conditions. However, under pathophysiological conditions, thrombin-mediated platelet activation plays a major role in arterial thrombosis.[5] In addition, cellular activation of thrombin is also known to play pro-inflammatory and proliferative roles in vascular disorders such as atherosclerosis and restenosis.

The cellular activity of thrombin is mediated via proteolytic activation of specific cell surface receptors known as protease activated receptors (PAR[a]).[6−10] There are four receptor subtypes known, PAR-1, PAR-3, and PAR-4, which are activated by thrombin, and PAR-2 which is activated by tryptase.[11] PAR-1 (also known as the thrombin receptor) is the most important of these receptors and is the most prevalent on human and primate platelets; PAR-4 is also present on human platelets but is activated only at very high thrombin concentrations and is hypothesized to be a rescue receptor that becomes activated in the event of a serious vascular lesion.

**Scheme 1.** Thrombin Receptor Antagonists Based on (+)-Himbacine




The thrombin receptor (PAR-1) belongs to the super family of seven transmembrane G-protein coupled receptors. The mechanism by which thrombin activates PAR-1 (discovered by Coughlin's group in 1991) is unique. Thrombin binds to PAR-1 through its exo-anion binding site. Cleavage of the extracellular domain at Arg[41]-Ser[42] reveals an amino terminus that then binds intramolecularly to the receptor.[12−14] Thrombin receptor activating peptides (TRAPs),[15] designed to mimic the amino terminus of the activated receptor, have been shown to elicit functional agonist responses such as platelet aggregation.

By virtue of the cellular action of thrombin, it has been hypothesized that a thrombin receptor antagonist may be useful in the treatment of disorders such as arterial thrombosis, atherosclerosis, and restenosis. Because a thrombin receptor (PAR-1) antagonist would only target the cellular effects of thrombin, while sparing its fibrin-generating property, such an agent could have a significant advantage in safety with regard to bleeding side effects over current antithrombotic therapies.[16−18]

We have recently reported the discovery of a potent thrombin receptor antagonist **1** (Sch 205831, Scheme 1)[19] based on the natural product himbacine. Compound **1** showed a $K_i$ value of 2.7 nM against the thrombin receptor and was highly active in several functional assays such as human platelet aggregation inhibition assay, calcium transient assay, and thymidine incor-

* Corresponding author. Tel: 908-740-2244. Fax: 908-740-7164. E-mail: martin.clasby@spcorp.com.
[†] Central Nervous System and Cardiovascular Chemical Research.
[‡] Cardiovascular and Metabolic Disease Research.
[§] Drug Metabolism and Pharmacokinetics Research.
[a] Abbreviations: ADP, adenosine diphosphate; hCASMC, human coronary artery smooth muscle cells; haTRAP, high-affinity thrombin receptor activating peptide; PAR, protease activated receptors; TRAP, thrombin receptor activating peptide.

10.1021/jm061043e CCC: $37.00 
Published on Web 12/13/2006

**Scheme 2.** C-Ring Hydroxylated Derivatives of **1**

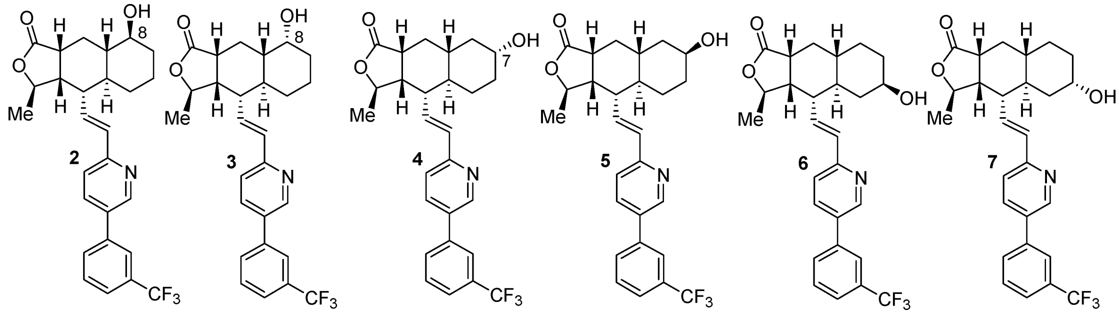


**Table 1.** Blood Levels of Compound **1** and Its Monohydroxylated Metabolites after Single and Multiple Oral Dosing in Rats at 300 mg/kg in 0.4% Methylcellulose

| compound | day | AUC (0−24 h) $\mu$g·h/mL | $C_{max}$ $\mu$g/mL |
|---|---|---|---|
| **1** | 1 | 35 | 29 |
| | 10 | 21 | 17 |
| **2** | 1 | 49 | 29 |
| | 10 | 150 | 79 |
| **4** | 1 | 93 | 58 |
| | 10 | 41 | 25 |
| **5** | 1 | 18 | 11 |
| | 10 | 81 | 47 |

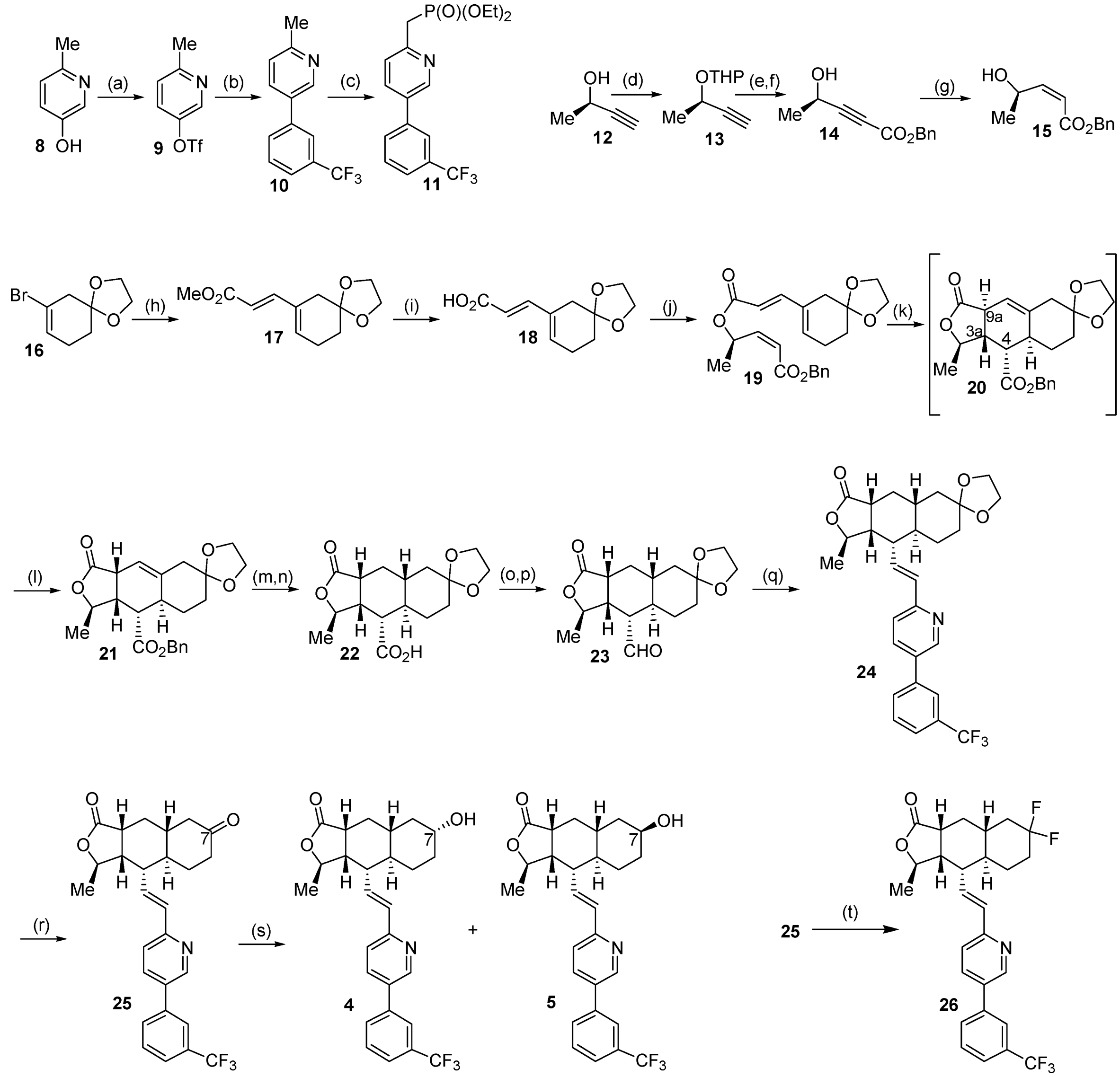
poration assay. This antagonist was highly selective for the PAR-1 receptor, had excellent oral bioavailability in rat and monkey models, and showed complete and sustained inhibition of platelet aggregation after oral administration in an ex-vivo cynomolgus monkey model.[20,21] Due to its excellent profile, compound **1** was selected for further development.

**Metabolic Profile of 1.** During the course of our biological profiling of **1**, the metabolism of $^{3}$H-**1** was evaluated in rat hepatocytes and cynomolgus monkeys following oral administration at 3 mg/kg in 0.4% methylcellulose. Plasma samples were taken from the monkeys at 6 and 24 h. In both species, one major and two minor hydroxylated metabolites were observed by LC-MS/MS analysis along with smaller amounts of dihydroxylated metabolites. In order to further characterize these metabolites, milligram quantities were generated by incubation of compound **1** with pregnenolone 16α-carbonitrile (PCN)-induced rat liver microsomes.[22] Subsequent NMR studies revealed that the major monohydroxylated metabolite was **2** (8$\beta$-OH-**1**), and the minor monohydroxylated metabolites were **4** (7α-OH-**1**) and **5** (7$\beta$-OH-**1**), the structures of which are shown in Scheme 2. The plasma concentration of **2** in monkeys was 160 ng/mL at 24 h and exceeded that of the parent (35 ng/mL of **1** at 24 h). The presence of high plasma levels of hydroxylated metabolites was a concern for us because this could indicate liver enzyme induction in addition to a prolonged plasma half-life. A 10-day enzyme induction study in rat was undertaken at daily oral doses of 100 mg/kg and 300 mg/kg. Fed male Sprague Dawley rats (four rats/ group) were dosed with compound **1** daily at 300 mg/kg and 100 mg/kg in 0.4% methylcellulose for 10 consecutive days. On day one and day ten, plasma concentrations of **1**, **2**, **4**, and **5** were measured at 1, 2, 4, 8, 12, and 24 h (the data is shown in Table 1). As we had seen earlier in monkeys, the level of compound **2** exceeded that of the parent **1**, and the in vivo levels of all three hydroxylated metabolites were high. The 100 and the 300 mg/kg group also showed a 10% and 34% increase in the liver/body weight ratio, respectively. Spectral cytochrome P450 (CYP) relative to the control group was increased by 80% and 99%, respectively, at the 100 and 300 mg/kg dose. Additionally, the 300 mg/kg group was found to have a 21-fold increase in the levels of the CYP2B enzyme after 10 days of dosing, and the CYP1A level was also modestly increased (3.6-fold). The elevation of hepatic cytochrome P450 (CYP) enzymes was associated with a 3-fold increase in the level of the 8 $\beta$-OH metabolite **2** on day 10 compared with day 1. A concomitant reduction in the level of parent between day 1 and day 10 was noted (AUC = 35 $\mu$g·h/mL on day 1 versus 21 $\mu$g·h/mL on day 10) which strongly suggested an autoinduction pattern for the hepatic enzymes. At 100 mg/kg, a small induction of CYP1A (1.8-fold) and CYP2B (2.5-fold) was noted. However, based on the plasma level of the drug at the efficacious dose of 3 mg/kg in cynomolgus monkey, the 100 mg/kg rat pharmacokinetic measurements gave an exposure multiple of only 5. Therefore, the development of **1** as a thrombin receptor antagonist was suspended due to expectation that adequate plasma exposure multiples of compound **1** would not be maintained in rodents in long term toxicological studies.

**Identification of a Replacement for Compound 1.** In an effort to identify a replacement candidate for compound **1** without enzyme induction liability, we adopted a 2-fold approach. The first approach was to explore C-ring hydroxylated derivatives (Scheme 2), including the known hydroxy metabolites. We reasoned that the enzyme induction was, in part, due to the lack of appropriate functional groups for conjugation and clearance. There have been several instances in the history of drug discovery research where a metabolite has served as an improved replacement for the initial drug candidate.[23−26] It was also important that such functional groups be situated at sterically unencumbered parts of the molecule to facilitate conjugation. The accumulation of 8-hydroxy metabolite **2** in rat plasma during the 10-day enzyme induction study suggested a slow clearance rate for this metabolite, perhaps due to steric hindrance around the hydroxyl group. A second approach was to selectively block the readily metabolized positions of the tricylic unit so that the molecule would undergo in vivo oxidation at alternate sites where it might be more easily conjugated and cleared.

**Synthesis of Targets.** The synthesis of the target molecules was carried out in a fashion similar to our earlier synthesis of tricyclic thrombin receptor antagonists,[19] as shown in Scheme 3. A notable exception in this case was that a protected ketone functionality was incorporated into the dienoic acid unit at an early stage of the synthesis for eventual elaboration to the hydroxy- or difluoro-substituted compounds. The other building blocks **11** and **15** were similar to the intermediates reported previously. Phosphonate **11** was synthesized in three steps from 2-methyl-5-hydroxypyridine **8** as shown in Scheme 3. Triflate formation followed by Suzuki coupling gave **10** which was converted into **11** by way of deprotonation followed by treatment with diethyl chlorophosphate. This synthesis of **11** can be applied to most 5-aryl 2-methylpyridines, and we have synthesized a number of phosphonates using this method. Alcohol **15**

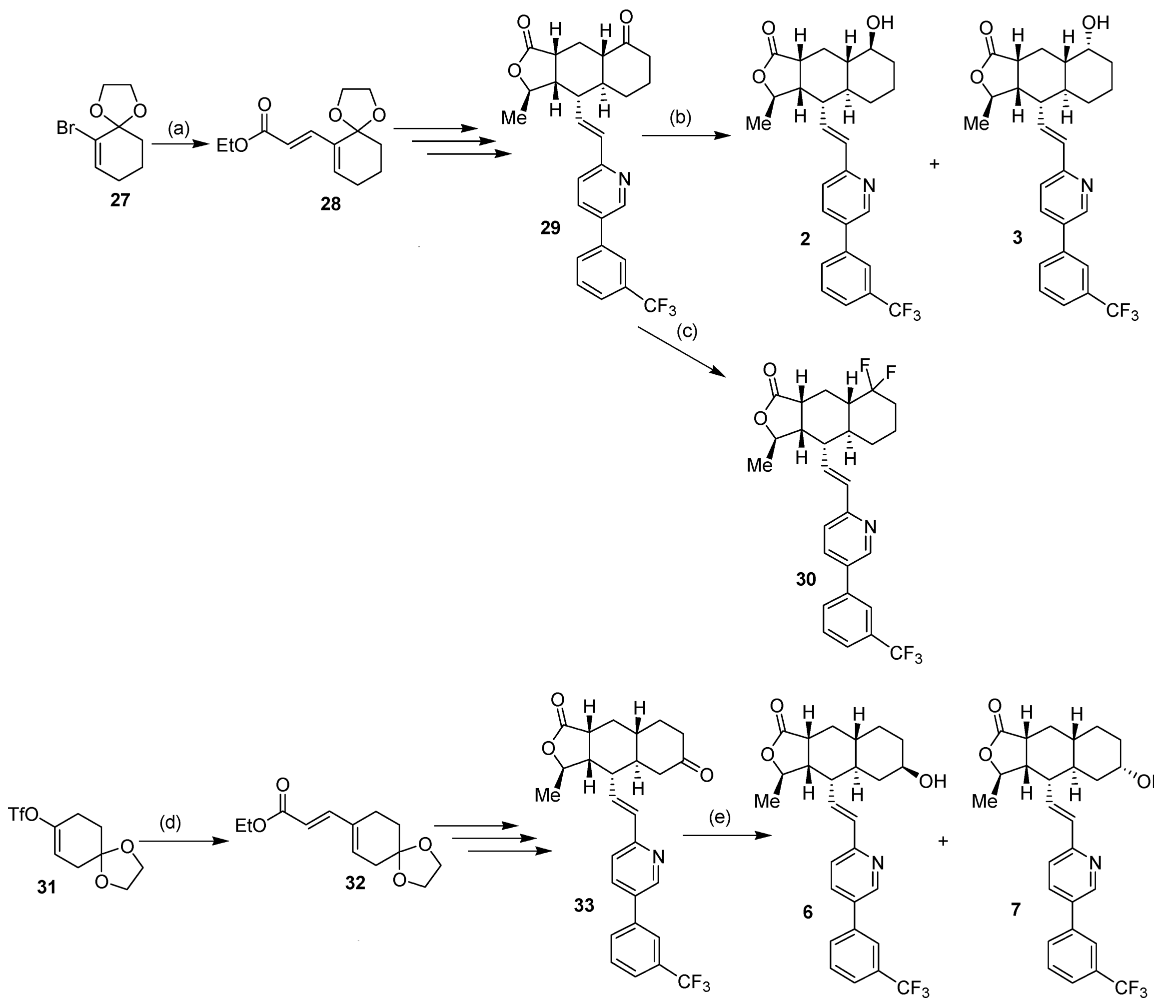
**Scheme 3.** Synthesis of Derivatives of **1**[a]



[a] Reagents and conditions: (a) $Tf_2O$, pyridine, 83%; (b) $ArB(OH)_2$, $K_2CO_3$, $Pd(Ph_3P)_4$, PhMe/ EtOH/$H_2O$, 82%; (c) LDA, $(EtO)_2POCl$, −78 °C, 85%; (d) DHP, TsOH, 100%; (e) BuLi, BnCOCl, THF, −78 °C; (f) TsOH, MeOH, 71%, two steps; (g) Lindlar catalyst, $H_2$ (1 atm), THF, 93%; (h) methyl acrylate, $(Ph_3P)_2PdCl_2$, DMF, 75 °C, 71%; (i) NaOH, MeOH, THF, 99%; (j) DCC, PPY, $CH_2Cl_2$, 77%; (k) xylene, 215 °C, 48%; (l) DBU, THF, 99%; (m) 10% Pd(C), $H_2$ (1 atm) EtOAc, 99%; (n) $PtO_2$, $H_2$; (1 atm), EtOAc, 99%; (o) $SOCl_2$, PhMe, 75 °C; (p) $Bu_3SnH$, $Pd(Ph_3P)_4$, PhMe, 0 °C, 48% two steps; (q) **11**, BuLi, THF, 0 °C then **23**, 83%; (r) HCl, acetone, 99%; (s) $NaBH_4$, EtOH, **4**, 70%, **5**, 15%; (t) DAST, DCE, 80 °C, 45%.

was synthesized in four steps. Protection of **12** with dihydropyran gave **13**, and deprotonation of the terminal alkyne and treatment with benzyl chloroformate followed by acid hydrolysis gave **14** which was reduced to **15** by hydrogenation over Lindlar catalyst. The absolute chirality of **15** was derived from use of enantiopure (*R*)-butynol **12**.[27] Dienoic acid **18** was synthesized in two steps from **16**.[28,29] Heck reaction of **16** with methyl acrylate gave **17** which was hydrolyzed to give **18**. DCC-mediated ester formation of **18** with **15** in the presence of 4-pyrrolidinopyridine gave **19** which was heated at 185 °C to give the intramolecular Diels−Alder (IMDA) adduct **20.** The absolute chirality of the stereogenic center of the IMDA precursor **19** is diastereospecifically transmitted to C3a of the tricyclic product **20** during Diels−Alder reaction by virtue of an allylic strain-induced facial selectivity of the dienophile in the IMDA transition state.[19] The remainder of the stereogenic centers in the middle ring of the IMDA adduct **20** can be envisioned as formed relative to C3a. For example, the relative stereochemistry between C-3a and C-9a is a function of the exo-selective transition state of the IMDA. The relative stereochemistry between C-3a and C-4 is imparted due to the cis-geometry of the dienophile. Epimerization of C9a in **20** with DBU produces the cis-lactone. At this point the tricyclic portion of the molecule was assembled and required only minor manipulation. The benzyl protecting group was removed by hydrogenolysis using 10% palladium on carbon as the catalyst, and diastereoselective hydrogenation of the internal double bond using Adams catalyst generates the C-8a stereogenecity in the required absolute and relative chirality. The key aldehyde **23** was synthesized in two steps from **22** via reduction of its acid chloride with tributyltin hydride in the presence of tetrakistriphenylphosphine palladium(0).[30] The phosphonate **11** was then coupled to the aldehyde **23** using Horner−Emmons conditions to give the ketal **24**. Eventual deprotection of the ketal and stereoselective reduction of the resulting ketone would produce the required C7 alcohol. The ketal moiety was removed under acidic hydrolysis conditions and the resulting ketone **25** reduced with sodium borohydride to give the 7-hydroxy metabolites **4** and **5** which were readily separable using standard silica gel chromatography. Additionally ketone **25** was converted to the

**Scheme 4.** Synthesis of 6- and 8-Substituted Targets[a]



[a] Reagents and conditions: (a) methyl acrylate, $(Ph_3P)_2PdCl_2$, DMF, 75 °C, 48%; (b) $NaBH_4$, EtOH, **2**, 27%, **3**, 55%; (c) DAST, DCE, 80 °C; (d) methyl acrylate, $(Ph_3P)_2PdCl_2$, DMF, 75 °C, 89%; (e) $NaBH_4$, EtOH.

7,7-difluoro derivative **26** via treatment with diethylaminosulfur trifluoride (DAST) (Scheme 3).

Similarly ketal protected precursors **27**[31] and **31** would eventually lead to the C-8 hydroxy derivatives **2** and **3** and the C-6 hydroxy compounds **6** and **7**, respectively (Scheme 4). The 8,8-difluoro compound **30** was synthesized in a manner similar to that of compound **25**. Comparison of the synthetic samples of **2**, **4**, and **5** with samples obtained from the *in vitro* metabolism studies gave independent confirmation of the structures of the three monohydroxylated metabolites.

A systematic study of the heteroaryl region of the monhydroxylated and gem-difluroinated thrombin receptor antagonists was also carried out. Toward this goal, Horner−Emmons reactions using appropriately substituted heteroaryl phosphonates were carried out on tricyclic aldehyde **23**.

## Results and Discussion

The hydroxyl and difluoro derivatives of **1** were screened in the *in vitro* binding assay using purified human platelet membranes as a PAR-1 source and [$^3$H]haTRAP as the ligand as previously described.[32] The PAR-1 binding results for the hydroxylated and fluorinated derivatives of **1** are given in Table **2**. Among the hydroxylated derivatives, the C-7 hydroxy compound **4** was the most potent ($IC_{50}$ = 17 nM). The C-8 hydroxylated compounds (**2** and **3**) and the C-7 hydroxy compound **5** showed slightly lower PAR-1 binding affinity, and

**Table 2.** Binding Data of Derivatives of **1**

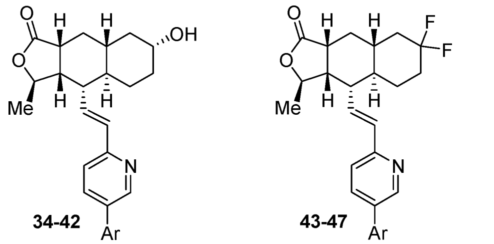
| compound | $IC_{50}$ (nM) ± SEM[a] |
|---|---|
| **2** | 41 ± 3.8 ($n$ = 3) |
| **3** | 28 ± 3 ($n$ = 4) |
| **4** | 17 ± 1.3 ($n$ = 8) |
| **5** | 23 ± 2.5 ($n$ = 4) |
| **6** | 481 ± 107 ($n$ = 4) |
| **7** | 845 ± 227 ($n$ = 4) |
| **26** | 37 ± 13 ($n$ = 2) |
| **30** | 53 ± 15 ($n$ = 5) |

[a] PAR-1 binding assay ligand: [$^3$H]haTRAP, 10 nM ($K_d$ = 15 nM).

the C-6 hydroxy compounds **6** and **7** were essentially inactive. Among the gem-difluorinated analogues tested the 8,8-difluoro derivative **30** had similar PAR-1 binding to the C-8 hydroxylated compounds (**2** and **3**); however, the 7,7-difluoro analogue **26** showed marginally more favorable PAR-1 binding.

An SAR study of the phenyl substitution in the C-7 hydroxy series and the 7,7-difluoro series confirmed our previous finding that ortho- and meta-substituted phenyl groups are favored over the para-substituted phenyl ring and that meta was similar to ortho. Selected SAR is shown in Table 3. Replacement of the 3-trifluoromethyl group in **4** with either chlorine (**37**) or fluorine (**34**) led to a slight loss in potency ($IC_{50}$ = 38 and 28 nM, respectively), whereas replacement with a methyl group (**38**) led to a substantial loss in potency (147 nM). In the 7,7-difluorinated series the chlorinated derivatives **45** and **46** proved to be the most potent ($IC_{50}$ = 12, 11 nM, respectively).

**Table 3.** SAR of the Heteroaryl Region of Compounds **4** and **26**




| compound | Ar | $IC_{50}$ (nM), $n = 2 \pm$ SEM |
|---|---|---|
| **34** | 3-fluorophenyl | 28 ± 2 |
| **35** | 2-fluorophenyl | 72 ± 28 |
| **36** | 4-fluorophenyl | 829 ± 430 |
| **37** | 3-chlorophenyl | 38.5 ± 8.5 |
| **38** | 3-methylphenyl | 147 ± 5 |
| **39** | 2-methylphenyl | 70 ± 17 |
| **40** | 4-methylphenyl | 1374 ± 23 |
| **41** | 2,5-dichlorophenyl | 97 ± 1.5 |
| **42** | 2,3-dichlorophenyl | 16.5 ± 1.5 |
| **43** | 3-fluorophenyl | 15 ± 5 |
| **44** | 2-fluorophenyl | 28 ± 9 |
| **45** | 3-chlorophenyl | 12 ± 2.8 |
| **46** | 2-chlorophenyl | 11 ± 0.5 |
| **47** | 3-methylphenyl | 80 ± 20 |

[a] PAR-1 binding assay ligand: $[^3H]$haTRAP, 10 nM ($K_d = 15$ nM).

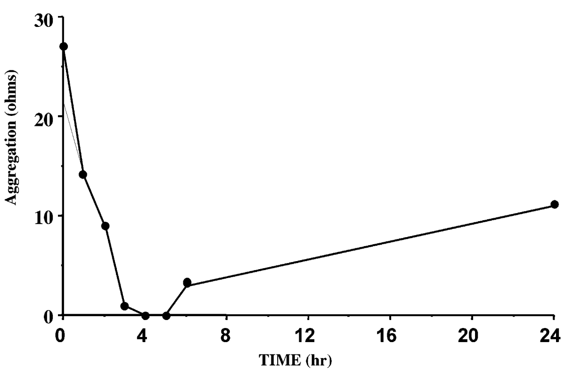



**Figure 1.** Ex-vivo platelet aggregation response after single dose (1 mg/kg, 20% HPBCD) of **4**.

In order to evaluate the *in vivo* activity of the difluorinated derivatives, representative compounds were selected for testing in the ex-vivo platelet aggregation inhibition assay in cynomolgus monkeys. Both compounds **26** and **45** were active only at a dose of 3 mg/kg in 20% HP$\beta$CD for 6 h (compared to 24 h for compound **1**). The 8,8-difluoro compound **30**, not surprisingly, was inactive when dosed. In general this series of compounds showed only moderate plasma levels in the rat (typically $AUC_{(0-6)} = 1200-1500$ ng•h/mL @ 10 mg/kg in 20% HP$\beta$CD). This is presumably due to poor oral absorption due to the low solubility of these compounds.[33]

From the in vitro data it can be seen that the 7-α-hydroxy metabolite **4** has potency ($IC_{50}$ =17 nM) similar to that of the parent compound **1** which had an $IC_{50}$ of 11 nM. In the pharmacokinetic studies conducted in rat, metabolites (**2**, **4**, and **5**) showed high plasma levels after oral administration ($AUC_{(0-6)}$ = 5755, 6130, and 3148 ng•h/mL, respectively). In the ex-vivo platelet aggregation assay in cynomolgus monkeys, compounds **4** and **5** were the most potent and showed complete inhibition of haTRAP-induced platelet aggregation at 1 mg/kg for 6 h, whereas compound **4** still showed 60% inhibition at the 24 h time point (Figure 1), additionally compound **4** showed blood levels that were consistent with this data (Figure 2). This is a 3-fold improvement over the parent compound **1**, which showed

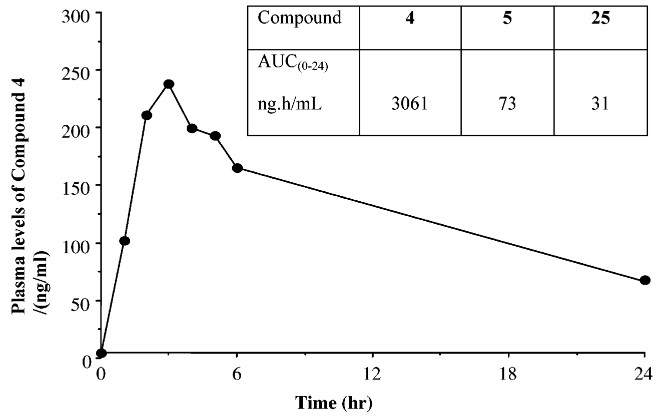



**Figure 2.** Blood level of **4** at various time points after single dose of 4 (1 mg/ kg, 20% HPBCD), in cynomolgus monkeys.

complete inhibition only at the 3 mg/kg dose. Interestingly, we noted that **5** underwent extensive metabolic transformation to **4** and the corresponding ketone **25** *in vivo* in cynomolgus monkeys, presumably an oxidation/reduction cycle via the ketone **25**. On the other hand, compound **4** was only minimally metabolized to **5** and **25** under the same experimental conditions in cynomolgus monkeys. The ketone **25** was equipotent to alcohols **4** and **5** in the cynomolgus monkey *ex vivo* model. Therefore, it is apparent that compound **4** is metabolically more stable in primates than its epimer **5**. However, this profile was found to be reversed in rats. When the alcohol **4** was dosed in rats, it underwent extensive metabolic conversion to **5** as measured by the plasma levels, whereas similar studies using **5** showed little conversion to **4**. Thus, the metabolic interconversion of **4** and **5** follows symmetrically opposite pathways in primates and rodents. This species-dependent metabolism was a concern for us; in particular we needed to know how these compounds would be metabolized in humans. In order determine this, we carried out a comparative study of compounds **4** and **5** in rat, monkey, and human hepatocytes. Upon incubation of **4** and **5** in rat and monkey hepatocytes, we obtained similar results to those seen *in vivo*; in cynomolgus monkeys, **4** was the preferred epimer while in rats **5** was the preferred epimer. A similar human hepatocyte incubation study also was undertaken to assess the potential for conversion of **4** to **5**. Upon incubation of compounds **4** and **5** in human hepatocytes, we obtained results similar to the monkey hepatocytes with **4** undergoing very little conversion to **5**, and **5** undergoing substantial interconversion to **4**. This correlation between the human and cynomolgus monkey metabolism gave us further assurance to the validity of using a cynomolgus monkey ex-vivo platelet aggregation assay as the pharmacodynamic model.

**Profile of 4.** Since compound **4** showed the best characteristics in terms of PAR-1 affinity, ex-vivo potency, and pharmacokinetics, it was selected for further studies. It binds competitively to PAR-1 on human platelet membranes with a $K_i$ of 11 nM against $[^3H]$haTRAP. Scatchard plots of saturation binding of $[^3H]$haTRAP in the presence and absence of **4** as consistent with a competitive inhibition of the radioligand binding to PAR-1. Compound **4** appears to dissociate very slowly from the receptor; compound **4** was preincubated with platelet membranes, and once saturation binding was achieved, the excess compound was washed out. Inhibition of binding of $[^3H]$haTRAP was observed for 3 h after the washout, thus indicating slow dissociation of compound **4** from the receptor. Slow dissociation from the receptor is thought to be critical in determining antagonist efficacy.[16,18,19]

Compound **4** has been tested for its ability to inhibit thrombin-mediated activation of calcium transients in human coronary smooth muscle cell (hCASMC). Intracellular calcium concentration was measured using a fluorescent calcium dye (Fluo-3) and a fluoremetric imaging plate reader. Thrombin elevated the intracellular calcium concentration in a dose-dependent manner with an $EC_{50}$ of 0.9 nM. Compound **4** completely inhibited this effect in a dose-dependent manner with a $K_i$ = 85 nM. When the smooth muscle cells were treated with **4**, no elevation of calcium levels was observed, indicating that **4** does not possess agonist activity.

PAR-1 activation is known to be mitogenic in smooth muscle cells.[16,18] Arterial smooth muscle cell proliferation is a key event in the formation of arterial lesions and restenosis that often occurs following percutaneous coronary artery interventions (PCI). The potential utility of a PAR-1 antagonist to inhibit these processes can be assessed *in vitro* by measuring thrombin-induced incorporation of thymidine in hCASMC. Compound **4** completely inhibited the thrombin-stimulated [$^3$H]thymidine incorporation with an apparent $K_i$ of 22 nM. This data suggests that **4** should inhibit arterial smooth muscle cell proliferation and therefore has potential utility as an antirestinosis agent in addition to its promising antiplatelet effects.

To characterize the antiplatelet effects of antagonist **4**, it was preincubated for 1 h with washed human platelets obtained from healthy subjects who had been aspirin-free for 7 days. The preincubation is required because, in addition to a slow disassociation-rate, **4** has a slow association rate. Antagonist **4** completely inhibited the aggregation response of the washed platelets to 0.3 $\mu$M of haTRAP with an $IC_{50}$ of 60 ± 10 nM. Additionally, **4** does not block the aggregation response of platelets to ADP, indicating that this activity is specific to inhibition of PAR-1. As mentioned previously, **4** showed complete inhibition of platelet aggregation in the ex-vivo model in cynomolgus monkeys for up to 6 h with 60% inhibition observed at 24 h. This was a 3-fold improvement in potency in activity in this model over **1** when dosed similarly.

Compound **4** shows good fasted oral absorption (92%) and bioavailability (89%) in cynomolgus monkeys. The bioavailability was not affected by the presence of food. The major metabolite in cynomolgus monkeys was the glucoronide, which was primarily excreted in bile. Compound **4** did not inhibit cytochrome P450's 2D6, 3A4, 2C9, or 2C19 in human liver microsomes at concentrations up to 10 $\mu$M. Enzyme induction studies of **4** in rats over 8 days showed a weak to moderate amount of enzyme induction of cytocrome P450s 1A and 2B at daily doses of 10 and 30 mg/kg. At both 10 and 30 mg/kg dose the change in body weight relative to control was not significant. We observed improved multiples for the parent and active metabolites in cynomolgus monkeys. This multiple was 21-fold at the 10 mg/kg dose and 36-fold at the 10 mg/kg. Additionally, contrary to compound **1**, no progressive reduction of the plasma level of the parent was noted at the 30 mg/kg dose (21.7 $\mu$g•h/mL on day 1 vs 21.3 $\mu$g•h/mL on day 8). This study indicates that it would be feasible to conduct a chronic drug safety study in rats. Other studies[34] have shown that **4** did not induce changes in gastrointestinal, renal, cardiovascular, respiratory, or central nervous system function.

## Summary

In summary, we have used a metabolism-based approach to identify compound **4** as an excellent replacement candidate for the prototypical thrombin receptor antagonist **1** which encountered development issues due to rat specific CYP-2B enzyme induction. A synthetic method was developed that allowed for the incorporation of 6-, 7-, and 8-hydroxy substitution as well as difluoro substitution. In addition we developed a method to allow variation in the biaryl moiety via palladium(0) chemistry.

The 7-hydroxy metabolite **4** is highly potent with a $K_i$ of 11 nM and showed 3-fold greater oral poptency in a cynomolgus monkey ex-vivo platelet aggregation inhibition model than its prototype **1**. In a 10-day multiple dosing rat enzyme induction model, compound **4** and its active metabolites showed excellent exposure multiples based on the plasma levels in cynomolgus monkey at the efficacious dose of 1 mg/kg. Additionally, these compounds showed a slow dissociation rate from PAR-1 receptor which is beneficial in competing against the tethered ligand mechanism.[16] Compound **4** did not affect clotting parameters, confirming that its mode of mechanism is not by active site inhibition of thrombin.

## Experimental Section

**General Comments.** Melting points were taken on a Thomas-Hoover or Mel-Temp II melting point apparatus and are uncorrected. Chromatography was performed over Universal Scientific or Selecto Scientific flash silica gel (particle size 32−63 $\mu$m). $^1$H NMR spectra were determined on a Gemini 400 MHz instrument using either $Me_4Si$ or residual solvent signal as internal standards. Rotations were determined on a Rudolph Autopol III or Perkin-Elmer 243B Polarimeter with concentration expressed in milligrams per 1 mL. Mass spectra were obtained on VG-ZAB-SE, Extrel-401, HP-MS Engine, JEOL HX-110, Sciex API 100, or Sciex API 150 mass spectrometer. Elemental analyses were determined by the Physical-Analytical Department of Schering-Plough Research Institute using either CEC 240-HA, CEC CE-440, or Fisons EA 1108 CHNS elemental analyzers and are within 0.4% of the theoretical value unless otherwise noted. The conditions for the LC/MS analyses in the preparations and examples below are as follows: 5 min gradient from 10% → 95% $CH_3CN/H_2O$ with 0.05% TFA, then 2 min isocratic at 95% $CH_3CN/H_2O$ with 0.05% TFA, 1.0 mL/min flow rate on a MAC-MOD ACE5 C18 column (4.6 × 50 mm). (*R*)-Butynol (ee > 97%) was purchased from DSM Fine Chemicals, 217 Rt. 46W, Saddle Brook, NJ 07663.

**Trifluoromethanesulfonic Acid 6-Methylpyridin-3-yl Ester (9).** Triflic anhydride (46 mL, 0.275 mol) was added dropwise to a stirred solution of 6-methylpyridin-3-ol **8** (10 g, 0.092 mol) in pyridine (200 mL) at 0 °C and stirred at 0 °C to room temperature for 16 h. The mixture was poured into ice−water (300 mL) and extracted with ether. The ether layer was washed with water (2 × 150 mL) and brine, dried ($MgSO_4$), and concentrated *in vacuo* to give **9** (18.7 g, 83%) as a brown oil. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.67 (s, 3H), 7.32 (d, 1H, $J$ = 8.5 Hz), 7.57 (dd, 1H, $J$ = 8.6, 2.8 Hz), 8.53 (d, 1H, $J$ = 2.8 Hz); MS (ESI) *m*/*z* 242 ($MH^+$, 100%).

**2-Methyl-5-(3-trifluoromethylphenyl)pyridine (10).** To a solution of pyridine **9** (8.5 g, 34.5 mmol) and 3-trifluoromethylphenylboronic acid (10 g, 55 mmol) in toluene (100 mL) were added EtOH (25 mL), $K_2CO_3$ (14.3 g, 104 mmol) in $H_2O$ (50 mL), and $Pd(PPh_3)_4$ (400 mg, 0.345 mmol). The mixture was heated in a closed pressure tube under argon at 120 °C for 16 h. The mixture was diluted with EtOAc, washed with 5% NaOH and brine, dried ($MgSO_4$), and concentrated *in vacuo*. Flash chromatography of the residue on a silica gel column with EtOAc−hexane (10:90 then 20:80) as eluent gave **10** (6.7 g, 82%) as yellow solids. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.68 (s, 3H), 7.32 (d, 1H, $J$ = 8 Hz), 7.62−7.90 (m, 5H), 8.79 (d, 1H, $J$ = 2 Hz).

**[5-(3-Trifluoromethylphenyl)pyridin-2-ylmethyl]phosphonic Acid Diethyl Ester (11).** Compound **10** (4 g, 0.0146 mol) and diisopropylamine (2.28 mL, 1.1 equiv) were dissolved in THF (73 mL) and cooled to −78 °C with stirring. *n*-Butyllithium (12.28 mL of a 2.5 M solution in hexanes, 2.1 equiv) was added dropwise, and after 20 min diethyl chlorophosphate (2.11 mL, 1 equiv) was added. After a further 20 min, the mixture was allowed to warm to rt. Ammonium chloride solution (saturated) was added and the mixture extracted with ethyl acetate. The organic extracts were dried (magnesium sulfate), concentrated, and chromatographed ($SiO_2$, 1:1 hexane/ethyl acetate to 100% ethyl acetate) to give **11** (3.9 g, 71%) as a tan oil. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.36 (t, 6H, $J$ = 7 Hz), 3.56 (d, 2H, $J$ = 22 Hz), 4.19 (dq, 4H, $J$ = 7, 7 Hz), 7.58−7.96 (m, 6H), 8.84 (d, 1H, $J$ = 2 Hz); MS (FAB) *m/z* 374 ($MH^+$, 100%);

**(2*R*)-2-(1-Methylprop-2-ynyloxy)tetrahydropyran (13).** (2*R*)-3-butynol **12** (15 mL, 0.204 mol) and 3,4-dihydro-2*H*-pyran (26.1 mL, 1 equiv) were stirred at 0 °C. To this mixture was added *p*-toluenesulfonic acid (monohydrate) (0.38 g, 5 mol %) and the mixture stirred for a further 2 h. EtOAc (319 mL) and $NaHCO_3$ (1.6 g) were added, and after another 1 h the mixture was filtered and concentrated. Chromatography ($SiO_2$, 19:1 hexane/EtOAc) gave 31.49 g (100%) of **13** as a mixture of diastereomers. $^1$H NMR (major diastereomer) (400 MHz, $CDCl_3$) $\delta$ 1.54 (d, $J$ = 7.5 Hz, 3H), 1.55-2.0 (m, 6H), 2.42 (s, 1H), 3.56 (m, 1H), 3.88 (m, 1H), 4.60 (br q, $J$ = 7.5 Hz, 1H), 5.00 (t, $J$ = 5.0 Hz, 1H).

**(2*R*)-4-Hydroxypent-2-ynoic Acid Benzyl Ester (14).** A solution of compound **13** (31.49 g, 0.204 mol) in THF (1 L) was cooled to −78 °C with stirring. *n*-BuLi (97.8 mL, 2.5 M, 1.2 equiv) was added dropwise. After stirring for 20 min, benzyl chloroformate (35.1 mL, 1.2 equiv) was added and the reaction mixture was stirred at −78 °C for 2 h. The mixture was allowed to warm to room temperature, a saturated solution of $NH_4Cl$ was added, and the mixture was extracted with EtOAc. The organic extracts were dried over anhydrous $MgSO_4$, concentrated under reduced pressure, and then dissolved in methanol (2 L). DOWEX 50WX8-100 ion-exchange resin (60 g, prewashed with MeOH) was added, and the mixture was stirred at room temperature overnight. The mixture was filtered, concentrated, and chromatographed ($SiO_2$, 9:1 to 4:1 hexane/EtOAc) to give 29.9 g (71%) of **14**. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.55 (d, $J$ = 7.5 Hz, 3H), 4.70 (q, $J$ = 7.5 Hz, 1H), 5.27 (s, 2H), 7.44 (br s, 5H).

**(2*R*)-4-Hydroxypent-(*Z*)-2-enoic Acid Benzyl Ester (15).** Compound **14** (23.28 g, 0.114 mol) was dissolved in THF (232 mL). Lindlar's hydrogenation catalyst was added (3.48 g). The mixture was then placed under 1 atm pressure of $H_2$(g) and stirred for 2.5 h. The mixture was filtered and concentrated *in vacuo* to give **15** (22 g, 93%). $^1$H NMR ($CDCl_3$) $\delta$ 1.32 (d, $J$ = 6.5 Hz, 3H), 5.09 (m, 1H), 5.17 (s, 2H), 5.86 (d, $J$ = 11.7 Hz, 1H), 6.30 (dd, $J$ = 11.7, 7.0 Hz, 1H), 7.38 (s, 5H).

**3-(1,4-Dioxaspiro[4.5]dec-7-en-7-yl)acrylic Acid Ethyl Ester (17).** 7-Bromo-1,4-dioxaspiro[4.5]dec-7-ene **16**[28,29] (27.5 g, 0.1255 mol) was dissolved in DMF (400 mL), and methylacrylate (23 mL, 0.251 mol), triethylamine (52.25 mL, 0.3765 mol), and $Pd(Ph_3P)_2Cl_2$ (4.37 g, 5 mol %) were added successively. The mixture was heated at 75 °C for 16 h. The reaction was worked up by the addition of $NH_4Cl$ (sat.), extracted with ether, and dried ($MgSO_4$). The extracts were concentrated *in vacuo*, and the residue was chromatographed (9:1 to 4:1 hexane/EtOAc) to give 20 g (71%) of **17**. $^1$H NMR ($CDCl_3$) $\delta$ 1.78 (t, $J$ = 6.5 Hz, 2H), 2.38 (s, 2H), 2.44 (m, 2H), 3.74 (s, 3H), 4.0 (s, 4H), 5.73 (d, $J$ = 15 Hz, 1H), 6.17 (br s, 1H), 7.36 (d, $J$ = 15 Hz, 1H).

**3-(1,4-Dioxaspiro[4.5]dec-7-en-7-yl)acrylic Acid (18).** Compound **17** (20 g, 0.089 mol) was dissolved in a 1:1 mixture of THF/MeOH (520 mL total). 1 M NaOH solution (260 mL) was added slowly. The mixture was stirred for 4 h then water was added. The mixture was washed with ether, the aqueous layer was then acidified to pH 1 and extracted with EtOAc (×3), the combined extracts were dried ($MgSO_4$), and the solution was concentrated in *vacuo* to give 19 g (99%) of **18**. $^1$H NMR ($CDCl_3$) $\delta$ 1.79 (t, $J$ = 6.5 Hz, 2H), 2.40 (s, 2H), 2.46 (m, 2H), 4.01 (m, 4H), 5.73 (d, $J$ = 15.7 Hz, 1H), 6.23 (s, 1H), 7.41 (d, $J$ = 15.7 Hz, 1H).

**(1′*R*,3′a *R*,8′a*S*,9′*S*,9′a*R*)-1′,3′a,5′,7′,8′,8′a,9′,9′a-Octahydro-1′-methyl-3′-oxo-spiro[1,3-dioxolane-2,6′(3′*H*)-naphtho[2,3-*c*]furan]-9′-carboxylic Acid Phenylmethyl Ester (21).** Acid **18** (18 g, 0.0856 mol) was dissolved in $CH_2Cl_2$ (350 mL) and cooled to 0 °C. 1,3-Dicyclohexylcarbodiimide (23.23 g, 0.112 mol) was added, followed by 4-pyrrolidinopyridine (1.39 g, 0.0094 mol). After 5 min of stirring, a solution of **15** (22 g, 0.1067 mol) in $CH_2Cl_2$ (127 mL) was added over a 10 min period. The mixture was stirred at 0 °C for 2 h and at rt for 1 h. The mixture was then filtered and concentrated *in vacuo*, and column chromatography (9:1 to 4:1 hexane/EtOAc) gave 27 g of **19**. Compound **19** was dissolved in xylene (300 mL) and heated at 215 °C for 7 h. Column chromatography (9:1 to 4:1 to 2:1 hexane/EtOAc) gave 13.2 g of **20**. Compound **20** was dissolved in THF (264 mL), and DBU (4.9 mL, 0.033 mol) was added. The mixture was stirred for 1 h, diluted with EtOAc (500 mL), washed with $NH_4Cl$(sat.), dried ($MgSO_4$), concentrated *in vacuo*, filtered through a pad (1 in.) of $SiO_2$ (eluting with EtOAc), and concentrated *in vacuo* to give **21** (13 g, 48% from **18**). $^1$H NMR ($CDCl_3$) $\delta$ 1.10 (d, $J$ = 6.0 Hz, 3H), 1.2 (m, 1H), 1.65-1.85 (m, 2H), 1.92 (m, 1H), 2.35 (m, 1H), 2.47 (m, 1H), 2.59 (dd, $J$ = 10.75, 4.0 Hz, 1H), 2.70 (m, 1H), (q, $J$ = 2.5 Hz, 1H), 3.85-4.0 (m, 5H), 4.45 (m, 1H), 5.15 (AB quartet, $J$ = 12.0, 10.5 Hz, 2H), 5.36 (br s, 1H), 7.35 (s, 5H).

**(1′*R*,3′a*R*,4′a*R*,8′a*R*,9′*S*,9′a*S*)-Decahydro-1′-methyl-3′-oxo-spiro[1,3-dioxolane-2,6′(3′*H*)-naphtho[2,3-*c*]furan]-9′-carboxylic Acid (22).** Ester **21** (4.92 g, 0.0123 mol) was dissolved in EtOAc (250 mL), 10% palladium on carbon (492 mg) was added, and the mixture was stirred under 1 atm of $H_2$(g) for 1 h. The mixture was filtered through a pad of celite. $PtO_2$ (492 mg) was added to the filtrate and the mixture stirred for 16 h under 1 atm $H_2$(g). The mixture was then filtered and concentrated *in vacuo* to give 3.81 g (99%) of **22**. $^1$H NMR ($CDCl_3$) $\delta$ 1.25 (m, 2H), 1.35 (d, $J$ = 6.5 Hz, 3H), 1.3-1.5 (m, 3H), 1.6 (m, 1H), 1.7-1.95 (m, 3H), 2.5 (m, 1H), 2.58 (m, 1H), 2.68 (m, 1H), 3.95 (m, 5H), 4.69 (m, 1H).

**(1′*R*,3′a*R*,4′a*R*,8′a*R*,9'*S*,9′a*S*)-Decahydro-1′-methyl-3′-oxo-spiro[1,3-dioxolane-2,6′(3′*H*)-naphtho[2,3-*c*]furan]-9′-carboxaldehyde (23).** Acid **22** (1 g, 0.0032 mol) was dissolved in toluene (20 mL), thionyl chloride (1.25 mL) was added, and the mixture was heated at 80 °C for 16 h. The mixture was then concentrated *in vacuo*, dissolved in fresh toluene (16 mL) and cooled to 0 °C. $Pd(Ph_3P)_4$ (186 mg) was added, followed by tributyltinhydride (1.3 mL, 0.0048 mol). The mixture was stirred for 3 h, chromatographed (4:1 to 2.5:1 hexane/EtOAc) to give 450 mg (48%) of **23**. $^1$H NMR ($CDCl_3$) $\delta$ 1.24 (d, $J$ = 6.5 Hz, 3H), 1.0-1.9 (m, 10H), 2.48 (m, 1H), 2.6-2.7 (m, 2H), 3.87 (m, 4H), 4.54 (m, 1H), 9.70 (br s, 1H).

**(1′*R*,3a′*R*,4a′*R*,8a′*R*,9′*S*,9a′*S*)-Decahydro-1′-methyl-9′-[(*E*)-2-[5-[3-(trifluoromethyl)phenyl]-2-pyridinyl]ethenyl]-spiro[1,3-dioxolane-2,6′(3′*H*)-naphtho[2,3-*c*]furan]-3′-one (24).** Phosphonate **11** (1.14 g, 0.0030 mol) was dissolved in THF (10 mL) and cooled to 0 °C. *n*-BuLi (1.9 mL of 2.5 M solution in hexanes, 0.0029 mol) was added and the mixture stirred for 10 min. This solution was then added to a solution of aldehyde **23** (450 mg, 0.00153 mol) in THF (10 mL) at 0 °C. This mixture was stirred for 2 h and then $NH_4Cl$ (sat.) was added. The mixture was extracted (EtOAc), dried ($MgSO_4$), concentrated *in vacuo*, and then chromatographed (60/40 hexane/EtOAc) to give 650 mg (83%) of **24**. $^1$H NMR ($CDCl_3$) $\delta$ 1.12-1.55 (m, 6H), 1.43 (d, $J$ = 6 Hz, 3H), 1.78 (m, 2H), 1.79 (m, 1H), 1.96 (dd, $J$ = 6.5, 3.0 Hz, 1H), 2.9 (m, 2H), 2.70 (quintet, $J$ = 6. 5 Hz, 1H), 3.95 (m, 4H), 4.76 (m, 1H), 6.55 (d, $J$ = 15 5 Hz, 1H), 6.65 (m, 1H), 7.29 (d, $J$ = 8.0 Hz, 1H), 7.60 (t, $J$ = 7.5 Hz, 1H), 7.66 (d, $J$ = 7.5 Hz, 1H), 7.75 (d, $J$ = 7.5 Hz, 1H), 7.80 (s, 1H), 7.86 (d, $J$ = 8.0 Hz, 1H), 8.79 (s, 1H).

**(3*R*,3a*S*,4*S*,4a*R*,8a*R*,9a*R*)-Octahydro-3-methyl-4-[(*E*)-2-[5-[3-(trifluoromethyl)phenyl]-2-pyridinyl]ethenyl]naphtho[2,3-*c*]-furan-1,7(3*H*,4*H*)-dione (25).** Compound **24** (650 mg, 0.00126 mol) was dissolved in acetone (7.5 mL), and HCl (7.5 mL of a 1 M solution) was added. The mixture was heated at 50 °C for 16 h. $NaHCO_3$(sat.) was added and the mixture extracted with EtOAc. The combined extracts were dried ($MgSO_4$), concentrated *in vacuo*, and chromatographed (1:1 hexane/EtOAc) to give 590 mg (99%) of **25**. $^1$H NMR ($CDCl_3$) $\delta$ 1.2-1.5 (m, 2H), 1.47 (d, $J$ = 7.0 Hz, 3H), 1.65 (m, 2H), 2.08 (m, 2H), 2.10 (m, 2H), 2.3-2.5 (m, 4 H), 2.74 (quintet, $J$ = 6.5 Hz, 1H), 4.80 (m, 1H), 6.59 (d, $J$ = 6.5 Hz, 1H), 6.72 (m, 1H), 7.28 (d, $J$ = 8.0 Hz, 1H), 7.61 (t, $J$ = 7.5 Hz, 1H), 7.66 (d, $J$ = 7.5 Hz, 1H), 7.76 (d, $J$ = 7.5 Hz, 1H), 7.81 (s, 1H), 7.87 (d, $J$ = 8.0 Hz, 1H), 8.80 (s, 1H); MS (CI) *m/z* 470 ($MH^+$, 100%); $[\alpha]_D^{20}$ −15.7 (c 7.13 mg/mL, MeOH); Anal. ($C_{27}H_{26}F_3NO_3$**.**HCl) C, H, N.

**(3*R*,3a*S*,4*S*,4a*R*,7*R*,8a*R*,9a*R*)-Decahydro-7-hydroxy-3-methyl-4-[(*E*)-2-[5-[3-(trifluoromethyl)phenyl]-2-pyridinyl]ethenyl]naphtho[2,3-*c*]furan-1(3*H*)-one (4) and (3*R*,3a*S*,4*S*,4a*R*,7*S*,8a*R*,9a*R*)-Decahydro-7-hydroxy-3-methyl-4-[(*E*)-2-[5-[3-(trifluoromethyl)phenyl]-2-pyridinyl]ethenyl]naphtho[2,3-*c*]furan-1(3*H*)-one (5).** Compound **25** (100 mg, 0.000213 mol) was dissolved in EtOH (8 mL), and $NaBH_4$ (30 mg) was added. After 5 min, $NaHCO_3$ (sat.) was added and the mixture extracted with EtOAc. The extracts were dried ($MgSO_4$) and concentrated *in vacuo*. Purification by preparative TLC (47.5:47.5:5 hexane/EtOAc/MeOH) gave two isomers. The least polar isomer **5** (15 mg, 15%): $^1$H NMR ($CDCl_3$) $\delta$ 1.15-1.4 (m, 4H), 1.43 (d, $J = 6.0$ Hz, 3H), 1.5-1.7 (m, 3H), 1.75-1.95 (m, 3H), 2.35-2.5 (m, 2H), 2.72 (quintet, $J = 6.6$ Hz, 1H), 4.16 (br s, 1H), 4.75 (m, 1H), 5.46, $J = 15.5$ Hz, 1H), 6.65 (m, 1H), 7.29 (d, $J = 8.0$ Hz, 1H), 7.60 (t, $J = 8$ Hz, 1H), 7.66 (d, $J = 7.5$ Hz, 1H), 7.76 (d, $J = 7.5$ Hz, 1H), 7.80 (s, 1H), 7.85 (d, $J = 8.0$ Hz, 1H), 8.79 (s, 1H); MS (CI) *m/z* 472 ($MH^+$, 100%); Anal. ($C_{27}H_{28}F_3NO_3$·$0.3H_2O$) C, H, N. The most polar isomer **4** (70 mg, 70%): $^1$H NMR ($CDCl_3$) $\delta$ 0.93 (m, 1H), 1.06-1.4 (m, 5H), 1.43 (d, $J = 6.0$ Hz, 3H), 1.6 (m, 1H), 1.85-2.05 (m, 4H), 2.40 (m, 2H), 2.70 (quintet, $J = 6.5$ Hz, 1H), 3.64 (m, 1H), 4.75 (m, 1H), 6.55 (d, $J = 15.5$ Hz, 1H), 6.64 (m, 1H), 7.29 (d, $J = 8.0$ Hz, 1H), 7.60 (t, $J = 7.75$ Hz, 1H), 7.65 (d, $J = 7.5$ Hz, 1H), 7.75 (d, $J = 7.5$ Hz, 1H), 7.80 (s, 1H), 7.85 (d, $J = 8.0$ Hz, 1H), 8.79 (s, 1H); MS (CI) *m/z* 472 ($MH^+$, 100%); $[\alpha]_D^{20}$ +18.6 (c 3.38 mg/mL, MeOH); Anal. ($C_{27}H_{28}F_3NO_3$·HCl·$0.1H_2O$) C, H, N.

**(3*R*,3a*S*,4*S*,4a*R*,8a*R*,9a*R*)-7,7-Difluoro-decahydro-3-methyl-4-[(*E*)-2-[5-[3-(trifluoromethyl)phenyl]-2-pyridinyl]ethenyl]naphtho[2,3-*c*]furan-1(3*H*)-one (26).** Compound **25** (0.63 g, 0.00134 mol) was dissolved in dichloroethane (6 mL), Diethylaminosulfur trifluoride (DAST) (0.383 mL, 0.0029 mol, 2.16 equiv) was added and the mixture stirred at 80 °C for 1 h. Saturated $NaHCO_3$ solution was added and the mixture extracted with EtOAc. The organic layer was dried ($MgSO_4$) and concentrated and the residue purified by silica gel chromatography (1:4 EtOAc in hexanes) to give 300 mg of compound **26** (45%). $^1$H NMR ($CDCl_3$) $\delta$ 1.16-1.77 (m, 6H), 1.44 (d, $J = 6.0$ Hz, 3H), 1.89-2.19 (m, 4H), 2.35-2.47 (m, 2H), 2.73 (q, $J = 6.8$ Hz, 1H), 4.71-4.77 (m, 1H), 6.53-6.68 (m, 2H), 7.25-7.29 (m, 1H), 7.57-7.68 (m, 2H), 7.75 (d, $J = 7.6$ Hz, 1H), 7.80 (s, 1H), 7.84-7.88 (m, 1H), 8.80 (m, 1H), MS (CI) *m/z* 492 ($MH^+$, 100%); $[\alpha]_D^{20}$ +26.6 (c 6.05 mg/mL, MeOH); Anal. ($C_{27}H_{26}F_5NO_2$·HCl·$1.2H_2O$) C, H, N.

**3-(1,4-Dioxaspiro[4.5]dec-6-en-6-yl)acrylic Acid Methyl Ester (28).** Compound **27**[31] (33 g, 0.151 mol) was dissolved in DMF (400 mL), and methyl acrylate (28 mL, 0.31 mol, 2 equiv), triethylamine (65.2 mL, 0.468 mol, 3 equiv), and $Pd(Ph_3P)_2Cl_2$ (5.5 g, 5 mol %) were added successively. The mixture was heated at 80 °C for 16 h. The reaction was worked up by the addition of $NH_4Cl$ (sat), extracted with ether, and dried ($MgSO_4$). The extracts were concentrated *in vacuo* and the residue chromatographed (15% EtOAc in hexanes) to give 16 g of **28** (48%). $^1$H NMR ($CDCl_3$) $\delta$ 1.72-1.82 (m, 4H), 2.18-2.27 (m, 2H), 3.73 (s, 3H), 4.03-4.12 (m, 4H), 6.06 (d, $J = 15.2$ Hz, 1H), 6.45 (m, 1H), 7.26 (d, $J = 15.2$ Hz, 1H).

**(3*R*,3a*S*,4*S*,4a*S*,8*S*,8a*S*,9a*R*)-Decahydro-8-hydroxy-3-methyl-4-[(*E*)-2-[5-[3-(trifluoromethyl)phenyl]-2-pyridinyl]ethenyl]-naphtho[2,3-c]furan-1(3*H*)-one (2) and (3*R*,3a*S*,4*S*,4a*S*,8*R*,8a*S*,9a*R*)-Decahydro-8-hydroxy-3-methyl-4-[(*E*)-2-[5-[3-(trifluoromethyl)phenyl]-2-pyridinyl]ethenyl]naphtho[2,3-*c*]furan-1(3*H*)-one (3).** Compound **29** (1 g, 0.0021 mol) was dissolved in 1:1 MeOH/THF (160 mL), and $NaBH_4$ (161 mg, 0.0042 mol, 2 equiv) was added. After 10 min, $NH_4Cl$(sat.) was added and the mixture extracted with EtOAc. The extracts were dried ($MgSO_4$) and concentrated *in vacuo*. Purification by silica gel chromatography (40% EtOAc/hexanes) gave the following in order of elution: 270 mg of **2** (27%), $^1$H NMR ($CDCl_3$) $\delta$ 0.85-0.95 (m, 1H), 1.10-1.28 (m, 2H), 1.30-1.45 (m, 3H), 1.50 (d, $J = 6$ Hz, 3H), 1.80-1.90 (m, 2H), 2.04-2.12 (m, 1H), 2.41-2.45 (m, 1H), 2.47-2.53 (m, 1H), 2.61-2.66 (m, 1H), 2.72-2.77 (m, 1H), 3.35 (br m, 1H), 4.80 (m, 1H), 6.58-6.72 (m, 2H), 7.34 (d, $J = 8$ Hz, 1H), 7.65-7.75 (m, 2H), 7.82 (d, $J = 7.6$ Hz, 1H), 7.87 (s, 1H), 7.90 (d, $J = 8$ Hz, 1H), 8.85 (s, 1H), LCMS ($MH^+ = 472.3$) purity = 95%, and 550 mg of **3** (55%), $^1$H NMR ($CDCl_3$) $\delta$ 0.82-0.95 (m, 1H), 1.24-1.40 (m, 3H), 1.48 (d, $J = 6$ Hz, 3H), 1.70-1.95 (m, 6H), 2.38-2.48 (m, 2H), 2.76 (q, $J = 6.6$ Hz, 1H), 3.95 (br s, 1H), 4.85 (m, 1H), 6.58-6.72 (m, 2H), 7.36 (d, $J = 8.3$ Hz, 1H), 7.65-7.75 (m, 2H), 7.82 (d, $J = 7.6$ Hz, 1H), 7.87 (s, 1H), 7.90 (d, $J = 8.5$ Hz, 1H), 8.85 (s, 1H), LCMS ($MH^+ = 472.3$) purity = 100%.

**Trifluoromethanesulfonic Acid 1,4-Dioxa-spiro[4.5]dec-7-en-8-yl Ester (31).** To a solution of 1,4-cyclohexanedione monoethylene ketal (10 g, 64 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (21 g, 102 mmol) in $CH_2Cl_2$ (350 mL) at room temperature was added triflic anhydride (16 mL, 96 mmol) and stirred for 16 h. The mixture was washed with $NaHCO_3$ (sat.). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. Flash chromatography of the residue on a silica gel column with EtOAc−hexane (5−95 then 10−90) as eluent gave **31** (13.4 g, 72%) as a clear oil. $^1$H NMR ($CDCl_3$) $\delta$ 1.96 (t, $J = 6.6$ Hz, 2H), 2.37 (m, 2H), 2.59 (m, 2H), 4.05 (m, 4H), 5.72 (m, 1H).

**3-(1,4-Dioxa-spiro[4.5]dec-7-en-8-yl)acrylic Acid Methyl Ester (32).** To a solution of **31** (13 g, 46 mmol) in DMF (150 mL) were added methyl acrylate (8.4 mL, 92 mmol), $Et_3N$ (19 mL, 138 mmol), and $Pd(PPh_3)_2Cl_2$ (1.62 g, 2.3 mmol). The mixture was stirred at 75 °C for 10 h. The mixture was diluted with $NH_4Cl$ (sat.) and extracted with ether. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. Flash chromatography of the residue on a silica gel column with EtOAc−hexane (15−85) as eluent gave **32** (9.15 g, 89%) as a clear oil. $^1$H NMR ($CDCl_3$) $\delta$ 1.90 (t, $J = 6.5$ Hz, 2H), 2.44 (m, 2H), 2.51 (m, 2H), 3.80 (s, 3H), 4.05 (s, 4H), 5.85 (d, $J = 15$ Hz, 1H), 6.11 (br s, 1H), 7.36 (d, $J = 15$ Hz, 1H).

**(−)-(3*R*,3a*S*,4*S*,4a*R*,6*R*,8a*S*,9a*R*)-Decahydro-6-hydroxy-3-methyl-4-[(*E*)-2-[5-[3-(trifluoromethyl)phenyl]-2-pyridinyl]ethenyl]-naphtho[2,3-*c*]f uran-1(3*H*)-one (6).** $^1$H NMR ($CDCl_3$) $\delta$ 0.88-2.78 (m, 13H), 1.48 (d, $J = 6.0$ Hz, 3H), 3.67 (m, 1H), 4.78 (m, 1H), 6.64 (m, 2H), 7.33 (d, $J = 8.0$ Hz, 1H), 7.64-7.92 (m, 5H), 8.85 (s, 1H); MS (FAB) *m/z* 472 ($MH^+$, 100%); $[\alpha]_D^{20}$ -11.1 (c 4.13 mg/ mL, MeOH); Anal. ($C_{27}H_{28}F_3NO_3$·HCl·$0.6CH_2Cl_2$) C, H, N. (calcd 59.31, 5.45, 2.51; found 59.03, 5.72, 2.68).

**(+)-(3*R*,3a*S*,4*S*,4a*R*,6*S*,8a*S*,9a*R*)-Decahydro-6-hydroxy-3-methyl-4-[(*E*)-2-[5-[3-(trifluoromethyl)phenyl]-2-pyridinyl]ethenyl]-naphtho[2,3-*c*]furan-1(3*H*)-one (7).** $^1$H NMR ($CDCl_3$) $\delta$ 1.08-2.78 (m, 13H), 1.48 (d, $J = 6.0$ Hz, 3H), 4.21 (m, 1H), 4.85 (m, 1H), 6.63 (m, 2H), 7.37 (d, $J = 8.0$ Hz, 1H), 7.64-7.92 (m, 5H), 8.83 (s, 1H); MS (FAB) *m/z* 472 ($MH^+$, 100%); $[\alpha]_D^{20}$ +41.2 (c 4.05 mg/ mL, MeOH); Anal. ($C_{27}H_{28}F_3NO_3$·HCl·$0.75CH_2Cl_2$) C, H, N. (calcd 58.30, 5.38, 2.45; found 58.23, 5.80, 2.45).

**(3*R*,3a*S*,4*S*,4a*S*,8a*S*,9a*R*)-8,8-Difluoro-decahydro-3-methyl-4-[(*E*)-2-[ 5-[3-(trifluoromethyl)phenyl]-2-pyridinyl]ethenyl]naphtho[2,3-*c*]furan-1(3*H*)-one (30).** $^1$H NMR ($CDCl_3$) $\delta$ 0.84-0.94 (m, 1H), 1.44 (d, $J = 6.8$ Hz, 3H), 1.46-1.81 (m, 6H), 1.86-1.93 (m, 1H), 2.14-2.25 (m, 1H), 2.31-2.40 (m, 2H), 2.43-2.49 (m, 1H), 2.70 (q, $J = 6.0$ Hz, 1H), 4.72-4.79 (m, 1H), 6.55-6.66 (m, 2H), 7.29 (d, $J = 8$ Hz, 1H), 7.59-7.68 (m, 2H), 7.76 (d, $J = 8$ Hz, 1H), 7.81 (s, 1H), 7.83-7.89 (m, 1H), 8.80 (s, 1H), MS (CI) *m/z* 492, Anal. ($C_{27}H_{26}F_5NO_2$·HCl·$1.25H_2O$) C, H, N.

**(3*R*,3a*S*,4*S*,4a*R*,7*R*,8a*R*,9a*R*)-Decahydro-7-hydroxy-3-methyl-4-[(*E*)-2-[5-(3-fluorophenyl)-2-pyridinyl]ethenyl]naphtho[2,3-*c*]-furan-1(3*H*)-one (34).** $^1$H NMR ($CD_3OD$) $\delta$ 0.95 (m, 1H), 1.0-1.6 (m, 6H), 1.38 (d, $J = 6.0$ Hz, 3H), 1.75-2.0 (m, 3H), 2.40 (m, 2H), 2.73 (quintet, $J = 6.25$ Hz, 1H), 3.56 (m, 1H), 4.75 (m, 1H), 6.62 (m, 2H), 7.1-7.2 (m, 1H), 7.44 (d, $J = 13.2$ Hz, 1H), 7.48-7.52 (m, 2H), 7.56 (d, $J = 8.4$ Hz, 1H), 8.04 (dd, $J$= 8.4, 2.4 Hz, 1H), 8.74 (d, $J = 2.4$ Hz, 1H); MS (CI) *m/z* 422 ($MH^+$, 100%); $[\alpha]_D^{20}$ +31 (c 4.36 mg/mL, MeOH); Anal. ($C_{26}H_{28}FNO_3$·HCl) C, H, N.

**(3*R*,3a*S*,4*S*,4a*R*,7*R*,8a*R*,9a*R*)-Decahydro-7-hydroxy-3-methyl-4-[(*E*)-2-[5-(2-fluorophenyl)-2-pyridinyl]ethenyl]naphtho[2,3-*c*]-furan-1(3*H*)-one. (35).** $^1$H NMR ($CDCl_3$) $\delta$ 0.97 (m, 1H), 1.14-1.41 (m, 5H), 1.49 (d, $J = 6.0$ Hz, 3H), 1.69 (m, 1H), 1.9-2.1 (m, 4H), 2.40 (m, 2H), 2.75 (quintet, $J = 6.4$ Hz, 1H), 3.7 (m, 1H), 4.80 (m, 1H), 6.55-6.70 (m, 2H), 7.22-7.33 (m, 3H), 7.40-7.46 (m, 1H), 7.50 (t, $J = 7.7$ Hz, 1H), 7.89 (d, $J$= 8.0 Hz, 1H), 8.79 (s, 1H); MS (CI) *m/z* 422 (MH$^+$, 100%); $[\alpha]_D^{20}$ +33.4 (c 1.95 mg/mL, MeOH); Anal. ($C_{26}H_{28}FNO_3{\cdot}H_2O$) C, H, N.

**(3*R*,3a*S*,4*S*,4a*R*,7*R*,8a*R*,9a*R*)-Decahydro-7-hydroxy-3-methyl-4-[(*E*)-2-[5-(4-fluorophenyl)-2-pyridinyl]ethenyl]naphtho[2,3-*c*]furan-1(3*H*)-one (36).** $^1$H NMR ($CD_3OD$) $\delta$ 0.97 (m, 1H), 1.11 (m, 1H), 1.2-1.4 (m, 4H), 1.37 (d, *J* = 6.4 Hz, 3H), 1.79 (m, 1H), 1.87 (dd, *J* = 11.2, 4.8, 1H), 1.93 (br d, *J* = 12 Hz, 2H), 2.44-2.48 (m, 1H), 2.45-2.49 (m, 1H), 2.77 (quintet, *J* = 6.0 Hz, 1H), 3.59 (m, 1H), 4.80 (m, 1H), 6.80 (d, *J* = 16 Hz, 1H), 7.13 (dd, *J* = 16, 10.4 Hz, 1H), 7.33 (t, *J* = 8.8 Hz, 2H), 7.85 (m, 2H), 8.32 (d, *J* = 8.8 Hz, 1H), 8.77 (dd, *J* = 8.8, 1.6 Hz, 1H), 8.95 (s, 1H); MS (CI) *m/z* 422 (MH$^+$, 100%); $[\alpha]_D^{20}$ +30.6 (c 3.7 mg/mL, MeOH); Anal. ($C_{26}H_{28}FNO_3{\cdot}HCl$) C, H, N.

**(3*R*,3a*S*,4*S*,4a*R*,7*R*,8a*R*,9a*R*)-Decahydro-7-hydroxy-3-methyl-4-[(*E*)-2-[5-(3-chlorophenyl)-2-pyridinyl]ethenyl]naphtho[2,3-*c*]furan-1(3*H*)-one (37).** $^1$H NMR ($CDCl_3$) $\delta$ 0.9-1.03 (m, 2H), 1.1-1.4 (m, 4H), 1.49 (d, *J* = 6.4 Hz, 3H), 1.7 (br s, 1H), 1.8-2.1 (m, 4H), 2.43 (m, 2H), 2.75 (quintet, *J* = 6.4 Hz, 1H), 3.7 (m, 1H), 4.81 (m, 1H), 6.57-6.7 (m, 2H), 7.32 (d, *J* = 8 Hz, 1H), 7.41-7.53 (m, 3H), 7.61 (s, 1H), 7.88 (dd, *J* = 8.1, 2.2 Hz, 1H), 8.8 (s, 1H); MS (CI) *m/z* 438 (MH$^+$, 100%); Anal. ($C_{26}H_{28}ClNO_3{\cdot}1.5H_2O$) C, H, N.

**(3*R*,3a*S*,4*S*,4a*R*,7*R*,8a*R*,9a*R*)-Decahydro-7-hydroxy-3-methyl-4-[(*E*)-2-[5-(3-methylphenyl)-2-pyridinyl]ethenyl]naphtho[2,3-*c*]furan-1(3*H*)-one (38).** $^1$H NMR ($CDCl_3$) $\delta$ 0.92 (m, 1H), 1.05-1.35 (m, 5H), 1.45 (d, *J* = 6.2 Hz, 3H), 1.54 (m, 1H), 1.88-2.1 (m, 4H), 2.3-2.4 (m, 2H), 2.43 (s, 3H), 2.70 (quintet, *J* = 6.7 Hz, 1H), 3.65 (m, 1H), 4.75 (m, 1H), 6.55 (m, 2H), 7.2-7.26 (m, 2H), 7.36-7.38 (m, 3H), 7.82 (dd, *J* = 8.2, 2.3 Hz, 1H), 8.77 (d, *J* = 2 Hz, 1H); MS (CI) *m/z* 418 (MH$^+$, 100%); $[\alpha]_D^{20}$ +28.6 (c 4.8 mg/mL, MeOH); Anal. ($C_{27}H_{31}NO_3{\cdot}H_2O$) C, H, N.

**(3*R*,3a*S*,4*S*,4a*R*,7*R*,8a*R*,9a*R*)-Decahydro-7-hydroxy-3-methyl-4-[(*E*)-2-[5-(2-methylphenyl)-2-pyridinyl]ethenyl]naphtho[2,3-*c*]furan-1(3*H*)-one (39).** $^1$H NMR ($CD_3OD$) $\delta$ 0.92 (m, 1H), 1.1 (m, 1H), 1.15-1.37 (m, 4H), 1.39 (d, *J* = 6 Hz, 3H), 1.82-1.86 (m, 2H), 1.93-1.96 (m, 2H), 2.26 (s, 3H), 2.37-2.4 (m, 2H), 2.70 (quintet, *J* = 6.4 Hz, 1H), 3.55 (m, 1H), 4.85 (m, 1H), 6.6 (m, 2H), 7.2-7.31 (m, 4H), 7.53 (d, *J* = 8 Hz, 1H), 7.74 (dd, *J*= 8, 2 Hz, 1H), 8.42 (s, 1H); MS (CI) *m/z* 418 (MH$^+$, 100%); $[\alpha]_D^{20}$ +27.6 (c 3.94 mg/ mL, MeOH); Anal. ($C_{27}H_{31}NO_3{\cdot}HCl$) C, H, N.

**(3*R*,3a*S*,4*S*,4a*R*,7*R*,8a*R*,9a*R*)-Decahydro-7-hydroxy-3-methyl-4-[(*E*)-2-[5-(4-methylphenyl)-2-pyridinyl]ethenyl]naphtho[2,3-*c*]furan-1(3*H*)-one (40).** $^1$H NMR ($CDCl_3$) $\delta$ 0.97 (m, 1H), 1.11 (m, 1H), 1.22-1.4 (m, 4H), 1.37 (d, *J* = 6 Hz, 3H), 1.75-1.98 (m, 4H), 2.42 (s, 3H), 2.45-2.49 (m, 1H), 2.57 (m, 1H), 2.76 (quintet, *J* = 6 Hz, 1H), 3.58 (m, 1H), 4.91 (m, 1H), 6.81 (d, *J* = 16 Hz, 1H), 7.11 (dd, *J* = 16, 9.2 Hz, 1H), 7.4 (m, 2H), 7.7 (m, 2H), 8.29 (d, *J* = 8.4 Hz, 1H), 8.76 (d, *J*= 8.4 Hz, 1H), 8.93 (s, 1H); MS (CI) *m/z* 418 (MH$^+$, 100%); $[\alpha]_D^{20}$ +35 (c 3.15 mg/mL, MeOH); Anal. ($C_{27}H_{31}NO_3{\cdot}HCl{\cdot}0.2H_2O$) C, H, N.

**(3*R*,3a*S*,4*S*,4a*R*,7*R*,8a*R*,9a*R*)-Decahydro-7-hydroxy-3-methyl-4-[(*E*)-2-[5-(2,5-dichlorophenyl)-2-pyridinyl]ethenyl]naphtho-[2,3-*c*]furan-1(3*H*)-one (41).** $^1$H NMR ($CDCl_3$) $\delta$ 0.91 (m, 1H), 1.06-1.36 (m, 5H), 1.44 (d, *J* = 5.6 Hz, 3H), 1.86-2.04 (m, 4H), 2.32-2.41 (m, 2H), 2.70 (quintet, *J* = 6.4 Hz, 1H), 3.65 (m, 1H), 4.75 (m, 1H), 6.54 (d, *J* = 15.2 Hz, 1H), 6.63 (dd, *J* = 15.2, 9.6 Hz, 1H), 7.25-7.33 (m, 3H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.73 (dd, *J*= 8, 2 Hz, 1H), 8.6 (m, 1H); MS (CI) *m/z* 472 (MH$^+$, 100%); $[\alpha]_D^{20}$ +3.7 (c 13.9 mg/ mL, MeOH); Anal. ($C_{26}H_{27}Cl_2NO_3{\cdot}HCl{\cdot}0.3H_2O$) C, H, N.

**(3*R*,3a*S*,4*S*,4a*R*,7*R*,8a*R*,9a*R*)-Decahydro-7-hydroxy-3-methyl-4-[(*E*)-2-[5-(2,3-dichlorophenyl)-2-pyridinyl]ethenyl]naphtho-[2,3-*c*]furan-1(3*H*)-one (42).** $^1$H NMR ($CDCl_3$) $\delta$ 0.89 (m, 1H), 1.04-1.34 (m, 5H), 1.43 (d, *J* = 6 Hz, 3H), 1.85-2.02 (m, 4H), 2.30-2.39 (m, 2H), 2.68 (quintet, *J* = 6.8 Hz, 1H), 3.63 (m, 1H), 4.74 (m, 1H), 6.53 (d, *J* = 15.2 Hz, 1H), 6.60 (dd, *J* = 15.2, 8.8 Hz, 1H), 7.2-7.29 (m, 3H), 7.49 (d, *J* = 8 Hz, 1H), 7.71 (dd, *J*= 8.4, 2.4 Hz, 1H), 8.57 (m, 1H); MS (CI) *m/z* 472 (MH$^+$, 100%); $[\alpha]_D^{20}$ +21.6 (c 2.62 mg/ mL, MeOH); Anal. ($C_{26}H_{27}Cl_2NO_3{\cdot}HCl{\cdot}0.5H_2O$) C, H, N.

**(3*R*,3a*S*,4*S*,4a*R*,8a*R*,9a*R*)-7,7-Difluoro-decahydro-3-methyl-4-[(*E*)-2-[ 5-(3-fluorophenyl)-2-pyridinyl]ethenyl]naphtho[2,3-*c*]furan-1(3*H*)-one (43).** $^1$H NMR ($CDCl_3$) $\delta$ 1.16-1.77 (m, 6H), 1.44 (d, *J* = 6.0 Hz, 3H), 1.89-2.19 (m, 4H), 2.37-2.46 (m, 2H), 2.73 (q, *J* = 6.4 Hz, 1H), 4.74 (m, 1H), 6.54-6.65 (m, 2H), 7.07-7.12 (m, 1H), 7.24-7.28 (m, 2H), 7.34-7.38 (m, 1H), 7.41-7.47 (m, 1H), 7.82 (dd, *J* = 8, 2 Hz, 1H), 8.76 (m, 1H), MS (CI) *m/z* 442 (MH$^+$, 100%); $[\alpha]_D^{20}$ +25.1 (c 5.2 mg/mL, MeOH); Anal. ($C_{26}H_{26}F_3NO_2{\cdot}HCl{\cdot}1.3H_2O$) C, H, N.

**(3*R*,3a*S*,4*S*,4a*R*,8a*R*,9a*R*)-7,7-Difluoro-decahydro-3-methyl-4-[(E)-2-[ 5-(2-fluorophenyl)-2-pyridinyl] ethenyl]naphtho[2,3-*c*]furan-1(3*H*)-one (44).** $^1$H NMR ($CDCl_3$) $\delta$ 1.17-1.75 (m, 6H), 1.45 (d, *J* = 6.0 Hz, 3H), 1.88-2.18 (m, 4H), 2.38-2.46 (m, 2H), 2.73 (q, *J* = 6.8 Hz, 1H), 4.74 (m, 1H), 6.54-6.65 (m, 2H), 7.16-7.28 (m, 3H), 7.34-7.40 (m, 2H), 7.84 (d, *J* = 8.4 Hz, 1H), 8.674 (m, 1H), MS (CI) *m/z* 442 (MH$^+$, 100%); $[\alpha]_D^{20}$ +24.4 (c 4.5 mg/mL, MeOH); Anal. ($C_{26}H_{26}F_3NO_2{\cdot}HCl{\cdot}1.3H_2O$) C, H, N.

**(3*R*,3a*S*,4*S*,4a*R*,8a*R*,9a*R*)-7,7-Difluoro-decahydro-3-methyl-4-[(*E*)-2-[ 5-(3-chlorophenyl)-2-pyridinyl] ethenyl]naphtho[2,3-*c*]furan-1(3*H*)-one (45).** $^1$H NMR ($CDCl_3$) $\delta$ 1.16-1.76 (m, 6H), 1.44 (d, *J* = 6.0 Hz, 3H), 1.88-2.19 (m, 4H), 2.37-2.45 (m, 2H), 2.73 (q, *J* = 6.8 Hz, 1H), 4.74 (m, 1H), 6.53-6.65 (m, 2H), 7.25 (d, *J* = 7.2 Hz, 1H), 7.35-7.46 (m, 4H), 7.55 (m, 1H), 7.81 (dd, *J* = 8, 2.8 Hz, 1H), MS (CI) *m/z* 458 (MH$^+$, 100%); $[\alpha]_D^{20}$ +23.6 (c 4.2 mg/mL, MeOH); Anal. ($C_{26}H_{26}ClF_2NO_2{\cdot}HCl{\cdot}1.5H_2O$) C, H, N.

**(3*R*,3a*S*,4*S*,4a*R*,8a*R*,9a*R*)-7,7-Difluoro-decahydro-3-methyl-4-[(*E*)-2-[ 5-(2-chlorophenyl)-2-pyridinyl] ethenyl]naphtho[2,3-*c*]furan-1(3*H*)-one (46).** $^1$H NMR ($CDCl_3$) $\delta$ 1.17-1.72 (m, 6H), 1.46 (d, *J* = 6.0 Hz, 3H), 1.90-2.19 (m, 4H), 2.38-2.46 (m, 2H), 2.73 (q, *J* = 6.4 Hz, 1H), 4.75 (m, 1H), 6.55-6.65 (m, 2H), 7.26 (m, 1H), 7.34 (m, 3H), 7.49-7.51 (m, 1H), 7.77 (dd, *J* = 8, 2 Hz, 1H), 8.63 (d, *J* = 2.4 Hz, 1H), MS (CI) *m/z* 458 (MH$^+$, 100%); $[\alpha]_D^{20}$ +18.6 (c 4.8 mg/mL, MeOH); Anal. ($C_{26}H_{26}ClF_2NO_2{\cdot}HCl{\cdot}1.2H_2O$) C, H, N.

**(3*R*,3a*S*,4*S*,4a*R*,8a*R*,9a*R*)-7,7-Difluoro-decahydro-3-methyl-4-[(*E*)-2-[ 5-(3-methylphenyl)-2-pyridinyl] ethenyl]naphtho[2,3-*c*]furan-1(3*H*)-one (47).** $^1$H NMR ($CDCl_3$) $\delta$ 1.0-1.76 (m, 6H), 1.47 (d, *J* = 5.6 Hz, 3H), 1.93-2.19 (m, 4H), 2.30 (s, 3H), 2.40-2.45 (m, 2H), 2.73 (q, *J* = 6.8 Hz, 1H), 4.75 (m, 1H), 6.55-6.61 (m, 2H), 7.20-7.32 (m, 5H), 7.61-7.64 (m, 1H), 8.54 (d, *J* = 1.2 Hz, 1H), MS (CI) *m/z* 438 (MH$^+$, 100%); Anal. ($C_{27}H_{29}F_2NO_2{\cdot}HCl{\cdot}1.6H_2O$) C, H, N.

**Acknowledgment.** The authors thank Drs. Catherine Strader, John Piwinski, Michael Graziano, Ashit Ganguly, John Hunter, Ronald White, and Ted Sybertz for helpful discussions, Dr. Jesse Wong for scale-up of **22**, Drs. Birendra Pramanik and Pradip Das for mass spectral data, and Drs. T.-M. Chan and Mohindar Puar for NMR data.

**Supporting Information Available:** Microanalytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) Coleman, R. W.; Marder, V. J.; Salzman, E. W.; Hirsh, J. Overview of hemostasis. In *Hemostasis and Thrombosis: Basic Principles and Clinical Practice*; Coleman, R. W., Hirsch, J., Marder, V. J., Salzman, E. W., Eds.; Lippincott, Williams and Wilkins: Philadelphia, PA, 1994; pp 3−17.

(2) Davie, E. W.; Fujikawa, K.; Kisiel, W. The coagulation cascade: initiation, maintenance, and regulation. *Biochemistry* **1991**, *30*, 10363−10370.

(3) De Caterina, R.; Sicari, R. Cellular effects of thrombin: Pharmacology of the receptor(s) in various cell types and possible development of receptor antagonists. *Pharmacol. Res.* **1993**, *27*, 1−19.

(4) Shuman, M. A. Thrombin-cellular interactions. *Ann. N.Y. Acad. Sci.* **1986**, *485*, 228−239.

(5) Harker, L. A. Pathogenesis of thrombosis. In *Hematology*; Williams, W. J., Beutler, E., Erslev, A. J., Lictman, M. A., Eds.; McGraw Hill: New York, 1990; pp 1559−1568.

(6) Coughlin, S. R. Protease-activated Receptors. In *Handbook of Cell Signaling*; Bradshaw, R. A.; Dennis, E. A., Eds.; Elsevier: San Diego, 2004; Vol. 1, pp 167−171.

(7) Coughlin, S. R. Protease-Activated Receptors in the Cardiovascular System. *Cold Spring Harbor Symp. Quant. Biol.* **2002**, *67*, 197–208.

(8) Coughlin, S. R. Protease-Activated Receptors in Vascular Biology. *Thromb. Haemostasis* **2001**, *86*, 298–307.

(9) Grand, R. J. A.; Turnell, A. S.; Grabham, P. W. Cellular consequences of thrombin-receptor activation. *Biochem. J.* **1996**, *313*, 353–368.

(10) Coughlin, S. R. Protease-activated receptors in hemostasis, thrombosis and vascular biology. *J. Thromb. Haemostasis* **2005**, *3*, 1800–1814.

(11) Coughlin, S. R. How the protease thrombin talks to cells. *Proc. Natl. Acad. Sc. U.S.A.* **1999**, *96*, 11023–11027.

(12) Vu, T-K. H.; Hung, D. T.; Wheaton, V. I.; Coughlin, S.R. Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell* **1991**, *64*, 1057–1068.

(13) Hung, D. T.; Vu, T.-H.; Nelken, N. A.; Coughlin, S. R. Thrombin-induced events in non-platelet cells are mediated by the unique proteolytic mechanism established for the cloned platelet thrombin receptor. *J. Cell Biol.* **1992**, *116*, 827–832.

(14) Vu, T.-K. H.; Wheaton, V. I.; Hung, D. T.; Charo, I.; Coughlin, S. R. Domains specifying thrombin-receptor interaction. *Nature* **1991**, *353*, 674–677.

(15) Nanevicz, T.; Ishii, M.; Wang, L.; Chen, M.; Chen, J.; Turck, C. W.; Cohen, F. E.; Coughlin, S. R. Mechanisms of thrombin receptor agonist specificity. Chimeric receptors and complementary mutations identify an agonist recognition site. *J. Biol. Chem.* **1995**, *270*, 21619–21625.

(16) Chackalamannil, S. Thrombin Receptor (Protease-Activated Receptor-1) Antagonists as Potent Antithrombotic Agents with Strong Anti-platelet Effects. *J. Med. Chem.* **2006**, *49*, 5389–5403.

(17) Seiler, S. M.; Bernatowicz, M. S. Peptide-derived protease-activated receptor-1 (PAR-1) antagonists. *Curr. Med. Chem.; Cardiovasc. Hemat. Agents* **2003**, *1*, 13.

(18) Chackalamannil, S.; Xia, Y. Thrombin receptor (PAR-1) antagonists as novel antithrombotic agents. *Expert Opin. Ther. Pat.* **2006**, *16*, 493–505.

(19) Chackalamannil, S.; Xia, Y.; Greenlee, W. J.; Clasby, M.; Doller, D.; Tsai, H.; Asberom, T.; Czarniecki, M.; Ahn, H.-S.; Boykow, G.; Foster, C.; Agans-Fantuzzi, J.; Bryant, M.; Lau, J.; Chintala, M. Discovery of Potent Orally Active Thrombin Receptor (Protease-Activated Receptor 1) Antagonists as Novel Antithrombotic Agents. *J. Med. Chem.* **2005**, *48*, 5884–5887.

(20) Chackalamannil, S.; Asberom, T.; Xia, Y.; Doller, D.; Clasby, M. C.; Czarniecki, M. F. Preparation of himbacine analogs as thrombin receptor antagonists. U.S. Patent 6,063,847, May 16, 2000.

(21) Zhang, H.-C.; Derian, C. K.; Andrade-Gordon, P.; Hoekstra, W. J.; McComsey, D. F.; White, K. B. Poulter, B. L.; Addo, M. F.; Cheung, W.-M.; Damiano, B. P.; Oksenberg, D.; Reynolds, E. E.; Pandey, A.; Scarborough, R. M.; Maryanoff, B. E. Discovery and Optimization of A Novel Series of Thrombin Receptor (PAR-1) Antagonists: Potent, Selective Peptide Mimetics Based on Indole and Indazole Templates. *J. Med. Chem.* **2001**, *44*, 1021–1024.

(22) Kim, H.; Ahn, H.; Bryant, M.; Nomeir, A.; Chan, T.-M. Isolation and identification of the circulating major metabolites of the thrombin receptor antagonist SCH 205831 from rat liver microsomal incubations and rat bile using HPLC, LC/MS and LC/NMR. Manuscript in preparation.

(23) McClellan, K.; Jarvis, B. Desloratadine. *Drugs* **2001**, *61*, 789–796.

(24) Markham, A.; Wagstaff, A. J. Fexofenadine. *Drugs* **1998**, *55*, 269–274.

(25) Singh, S. S. Preclinical pharmacokinetics: an approach towards safer and efficacious drugs. *Curr. Drug Metab.* **2006**, *7*, 165–182.

(26) Nassar A-E.; F.; Kamel, A. M.; Clairmont, C. Improving the decision-making process in the structural modification of drug candidates: enhancing metabolic stability. *Drug Discovery Today* **2004**, *9*, 1020–1028.

(27) (*R*)-Butynol was purchased from DSM Fine Chemicals, 217 Rt. 46W., Saddle Brook, NJ 07663. This material had an enantiomeric excess greater than 97%. As described previously, due to the stereocontrol of the intramolecular Diels–Alder reaction,[19] we obtained compounds that were enantiopure (>99%) by HPLC (CHIRALPAK AD, CHIRALCEL OD).

(28) Lambert, J. B.; Liu, X. The $\beta$ -effect of silicon in the orthogonal geometry. *J. Organomet. Chem.* **1996**, *521*, 203–210.

(29) Shih, C.; Swenton, J. S. Use of protected $\beta$-bromocyclopentenones and $\beta$-bromocyclohexenones as $\beta$-acylvinyl anion equivalents. *J. Org. Chem.* **1982**, *47*, 2825–2832.

(30) Four, P.; Guibe, F. Palladium-catalyzed reaction of tributyltin hydride with acyl chlorides. A mild, selective, and general route to aldehydes. *J. Org. Chem.* **1981**, *46*, 4439–4445.

(31) Shih, C.; Fritzen, E. L.; Swenton, J. S. Photocycloaddition chemistry of 2-(trimethylsilyl)cyclopentenone and 5-(trimethylsilyl)uracil. The utility of a trimethylsilyl group as a removable directing group in photochemistry. *J. Org. Chem.* **1980**, *45*, 4462–4471.

(32) Ahn, H.-S.; Foster, C.; Boykow, G.; Arik, L.; Smith-Torhan, A.; Hesk, D.; Chatterjee, M. *Mol. Pharm.* **1997**, *51*, 350–356. Assays were carried out in duplicate. Compounds of high interest were assayed multiple times ($n > 5$, SEM $\pm$ 20%).

(33) Kinetic solubilities were measured for these compounds and were found to be ~5 $\mu$M

(34) Data not shown.


JM061043E