JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Synthesis of Biologically Active Piperidine Metabolites of Clopidogrel: Determination of Structure and Analyte Development

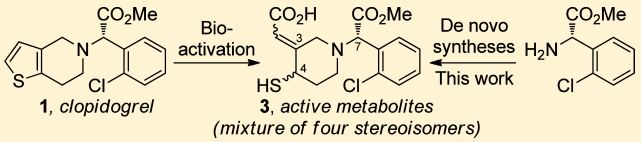
Scott A. Shaw,*[,†] Balu Balasubramanian,[†] Samuel Bonacorsi,[†] Janet Caceres Cortes,[†] Kevin Cao,[†] Bang-Chi Chen,[†] Jun Dai,[†] Carl Decicco,[†] Animesh Goswami,[†] Zhiwei Guo,[†] Ronald Hanson,[†] W. Griffith Humphreys,[†] Patrick Y. S. Lam,[†] Wenying Li,[†] Arvind Mathur,[†] Brad D. Maxwell,[†] Quentin Michaudel,[‡] Li Peng,[†] Andrew Pudzianowski,[†] Feng Qiu,[†] Shun Su,[‡] Dawn Sun,[†] Adrienne A. Tymiak,[†] Benjamin P. Vokits,[†] Bei Wang,[†] Ruth Wexler,[†] Dauh-Rurng Wu,[†] Yingru Zhang,[†] Rulin Zhao,[†] and Phil S. Baran[‡]

[†]Research and Development, Bristol-Myers Squibb Company, P.O. Box 5400, Princeton, New Jersey 08543-5400, United States

[‡]Department of Chemistry, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, California 92037, United States

Supporting Information

ABSTRACT: Clopidogrel is a prodrug anticoagulant with active metabolites that irreversibly inhibit the platelet surface GPCR $P2Y_{12}$ and thus inhibit platelet activation. However, gaining an understanding of patient response has been limited due to imprecise understanding of metabolite activity and stereochemistry, and a lack of acceptable analytes for quantifying in vivo metabolite formation. Methods for the production of all bioactive metabolites of clopidogrel, their stereochemical assignment, and the development of stable analytes via three conceptually orthogonal routes are disclosed.



INTRODUCTION

Tetrahydrothienopyridines, such as ticlopidine, clopidogrel (**1**, Plavix) and prasugrel (Effient, Figure 1), are prodrug antiplatelet agents that exert their action after metabolic activation to agents that bind to the $P2Y_{12}$ receptor. While there remains some uncertainty regarding the chemical and enzymatic steps of the bioactivation pathway, there is good agreement that the final products that potentiate platelet activity are 4-mercapto-3-piperidinylidene acetic acid derivatives.[1] The measurement of levels of these 4-mercapto-3-piperidinylidene acetic acid species (**3**, Figure 1, depicted for the active species of clopidogrel, **1**) in plasma is now regarded as the best marker of exposure and the parameter most closely related to pharmacodynamic activity.[2]

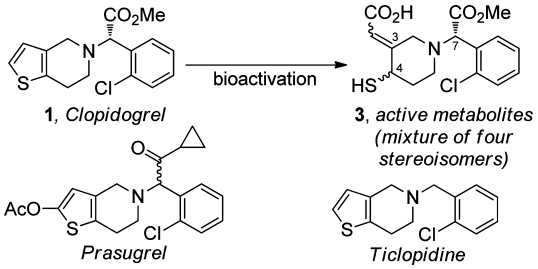


Figure 1. Thiophenopyridine antiplatelet agents.

The formation of **3** from clopidogrel is a multistep biochemical process with general agreement that the key steps are carried out by hepatic cytochrome-P450 (CYP) enzymes.[1d,3] The first step is oxidation at the 2-position of the thiophene ring to form 2-oxoclopidogrel (**2**, Figure 2). The subsequent cleavage of the thieno-ring is also through an oxidative mechanism, although the exact nature of the chemical intermediates involved is still under debate. Much of the controversy stems from the fact that the oxidation state is the same for the reactant and products of this transformation.

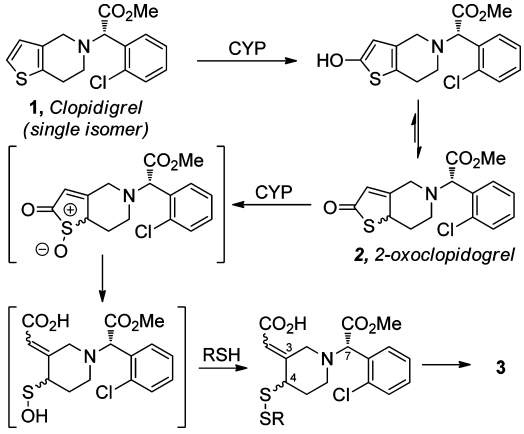


Figure 2. Proposed clopidogrel bioactivation pathway.

Received: April 3, 2015
Published: July 7, 2015

DOI: 10.1021/acs.joc.5b00632
J. Org. Chem. 2015, 80, 7019−7032

Recent studies have proposed that the cleavage step proceeds through a sulfenic acid intermediate that is reduced to give **3**.[4]

The reactions to form 2-oxoclopidogrel and to convert 2-oxoclopidogrel to **3** can be catalyzed by several CYP enzymes with varying kinetics, which has led to several conflicting reports regarding the predominant enzyme involved.[3,5] Besides the clear and important role for CYP2C19 in formation of **3**, there are other enzymes capable of clopidogrel bioactivation, with the best evidence being for CYP1A2, CYP2B6, and CYP3A4.[3] A landmark study by Shuldiner et al. using genome-wide association study (GWAS) analysis showed a dramatic impact of an allelic variation in the CYP2C19 gene on clopidogrel efficacy when studied in an Amish population.[6] The Shuldiner study, as well as other multiple, large longitudinal studies, have detected a relationship between CYP2C19 polymorphism and cardiovascular outcomes.[7] Besides the outcome studies, there are many biochemical and clinical lines of evidence that point to an important role for CYP2C19 in formation of **3** in antiplatelet response, and also a negative impact of the relatively common reduced function *2 and *3 alleles.[2,8]

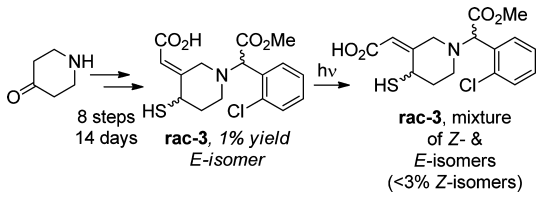
A publication in 2011 questioned the involvement of CYPs in the second activation step and proposed that an alternate PON1-mediated hydrolytic cleavage pathway was dominant in the ring-opening reaction.[9] Polymorphisms in PON1 enzymes were proposed to lead to reduced formation of **3**, altered platelet reactivity, and overall worse outcomes. However, multiple subsequent studies examining the PON1 enzymology in incubations with clopidogrel have found little evidence for the role of PON1 in the activation of clopidogrel or on outcomes of clopidogrel treatment.[10] While the PON1 enzyme is capable of hydrolyzing 2-oxoclopidogrel, studies have shown that the product is an inactive, isomeric thiol species.[11]

Part of the challenge of determining the stereochemical assignment and relative antiplatelet activity of each of the four stereoisomeric pharmacologically active metabolites of clopidogrel has been the difficulty in getting reasonable quantities of authentic, well-characterized **3** as discrete stereoisomers via chemical synthesis. The control of stereochemistry and the stability of intermediates and end products were the major challenges faced in devising and executing the new routes presented in this manuscript.

RESULTS AND DISCUSSION

Prior to our work, one reported synthesis of the proposed four stereoisomeric clopidogrel active metabolites was described (Scheme 1).[12,13] Fumitoshi et al. at Sankyo and Ube reported the preparation of metabolites **3** as part of their evaluation of cyclic amine antiplatelet agents. We evaluated the suitability of this route to satisfy our material needs and encountered a number of significant challenges. First, the synthesis is lengthy (eight steps over 2 weeks), involves several unstable intermediates and results in a reported 1% overall yield for the C-3 *E*-enoate isomers of **3**. Furthermore, accessing the *Z*-isomers requires photoisomerization of the C-3 *E*-enoate in 3% yield. Finally, this route does not afford the opportunity to control any of the stereoisomers including the C-7 phenylglycine moiety, and only the 7-*S* isomer possesses appreciable antiplatelet activity. In light of these challenges to obtain significant quantities[14] of analytical materials, we chose to examine three parallel, conceptually orthogonal approaches to prepare and assign the stereochemistry of all of the active clopidogrel metabolites and relevant analytical standards: (a) a biomimetic synthesis, (b) a stereoselective, stereodivergent approach, and (c) a stereoselective, chiral pool approach.

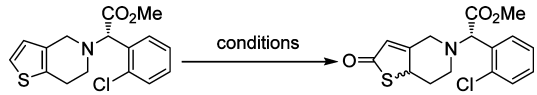
Scheme 1. Sankyo–Ube Metabolite Synthesis



Biomimetic Route Development. Our biomimetic approach began with examination of the discrete bioactivation steps involved in clopidogrel metabolism (Figure 2). Clopidogrel is activated in vivo in a two-step process beginning with oxidation of the thiophene ring by one of several cytochrome P450 enzymes to furnish thiophenone **2**. Subsequently, this thioester is hydrolyzed in a process believed to be mediated by a different subset of cytochrome P450 enzymes to furnish four stereoisomeric clopidogrel metabolites. Interestingly, while the stereochemistry of the C-3 olefin begins as exclusively the *Z*-isomer, the hydrolysis proceeds with concomitant isomerization of the olefin. A biomimetic approach was attractive due to our knowledge about the stability of the intermediates based on previous studies of clopidogrel bioactivation as well as on the metabolites isolated from patients.[1] Furthermore, in its idealized form this process would only require two steps from readily available clopidogrel to accomplish the synthesis of all the metabolites while controlling the C-7 phenylglycine stereocenter.

The biomimetic approach began by examining thiophene oxidation conditions (Scheme 2). Direct oxidation methods employing hydrogen peroxide/sodium carbonate or *m*CPBA were complicated by the presence of the basic piperidine amine and failed to furnish any of the desired thiophenone **2**, resulting in no conversion or decomposition, respectively. As an alternative to powerful electrophilic oxidants, direct lithiation of the thiophene either with *n*-butyllithium or LDA followed by treatment with trimethylborate and basic hydrogen peroxide was attempted but also failed to produce measurable quantities of **2**. Similarly, lithiation followed by treatment with Davis oxaziridine failed to produce **2**. Furthermore, clopidogrel (**1**) proved to be inert to standard Ir-mediated borylation

Scheme 2. Biomimetic Activation of Clopidogrel



1 **2**

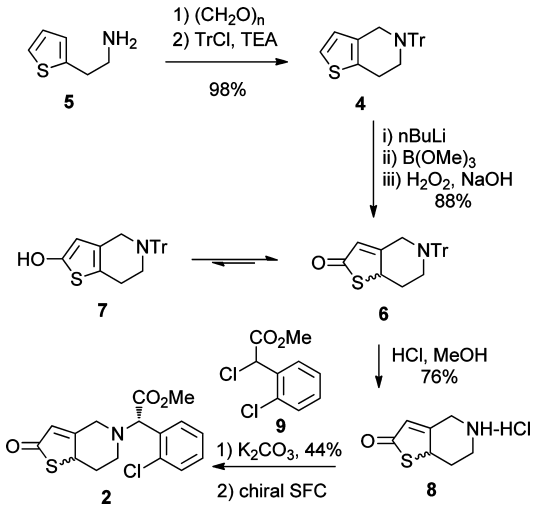
Entry	Reagents	Outcomes
1	*m*CPBA	decomposition
2	H_2O_2, Na_2CO_3	no reaction
3	i) nBuLi, $B(OMe)_3$ ii) H_2O_2, NaOH	decomposition
4	Ir-cat, $B_2(pin)_2$	no reaction
5	nBuLi; Davis oxaziridine	decomposition

DOI: 10.1021/acs.joc.5b00632
J. Org. Chem. 2015, 80, 7019–7032

conditions, suggesting that an alternative strategy would be required.

Reasoning that the difficulties in lithiating/oxidizing clopidogrel **1** could be due to the presence of the halophenylglycine methyl ester, we explored oxidation of protected thiophenopiperidine intermediate **4** (Scheme 3). This intermediate was prepared in two steps from commercially available 2-(thiophen-1′-yl)aminoethane (**5**) via treatment with paraformaldehyde and HCl followed by trityl protection. Treatment of thiophene **4** with *n*-butyllithium, trimethylborate, and hydrogen peroxide resulted in the efficient preparation of racemic thiophenone **6** in 88% isolated yield. Resolution of the enantiomers of **6** could be accomplished through supercritical fluid chromatography (SFC); however, unsurprisingly the resulting material epimerized readily upon standing, presumably via the intermediacy of aromatic thiophenone tautomer **7**.

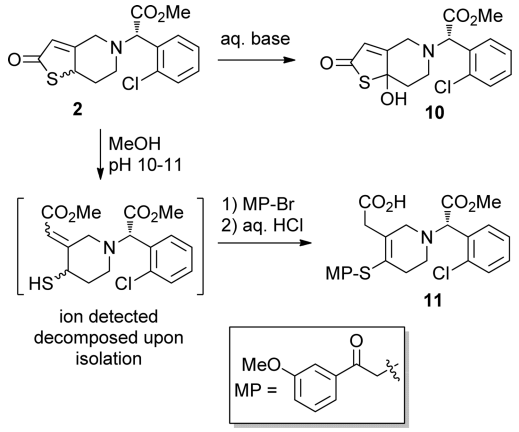
Scheme 3. Preparation of 2



Further elaboration to **2** was achieved by removal of the trityl group via treatment with HCl in MeOH. Treatment of the resulting amine **8** with alkyl halide **9** completed the synthesis of *rac*-2-oxoclopidogrel (**2**) as a 1:1 mixture of diastereomers. The resulting product was cleanly resolvable via chiral stationary phase SFC into four stereoisomers. Crystallization of one of the stereoisomers allowed the assignment of the absolute stereochemistry at C-7 by anomalous dispersion. This molecule exhibited diastereotopic protons in the ^{1}H NMR spectra, as well as peak interconversion during LC analysis to one of the other stereoisomeric structures. This demonstrated that the C-4 isomeric stereocenter was epimerizing similar to what was observed for thiophenone **6** and precluded separation of the C-4 isomers of **2** at this stage.

With the preparation of (7*S*)-**2** in hand, all that remained was determining conditions to effect the ring-opening of the thiophenone (Scheme 4). Attempts to hydrolyze the thiophenone with hydrochloric acid, similar to an analogous thioester solvolysis in the Sankyo−Ube synthesis, resulted only in decomposition. Treatment of **2** with a pH 10−11 water solution resulted exclusively in the formation of oxidized **10**. Presumably this arose via enolate formation followed by reaction with molecular oxygen. Rigorous degassing of reagents and performing the reaction under an inert atmosphere suppressed formation of **10**; however, none of the desired hydrolysis product was observed. In contrast, treatment of

Scheme 4. Solvolysis Studies on 2-Oxoclopidogrel



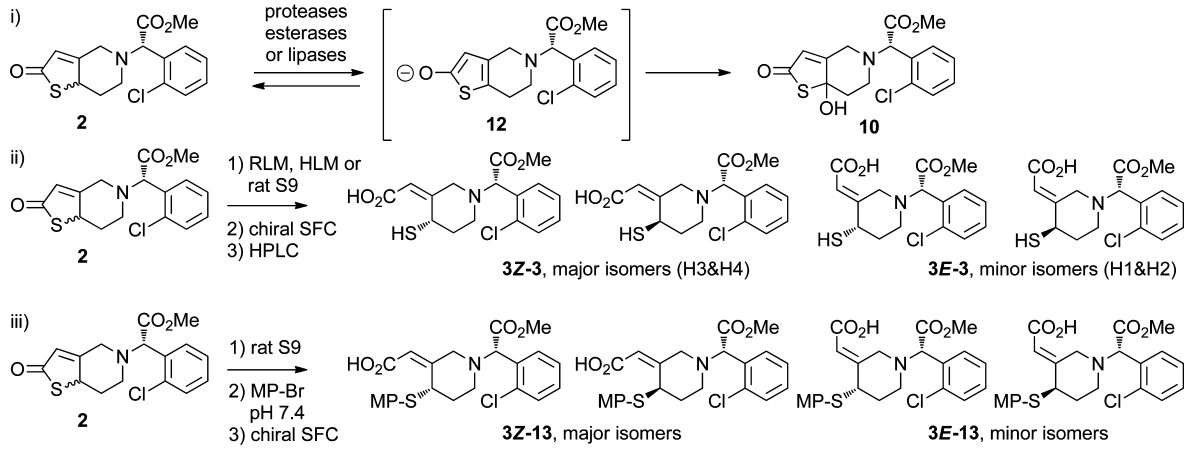
(7*S*)-**2** with a basic MeOH/aq pH 10−11 solution did produce an intermediate with a molecular ion corresponding to the expected solvolysis product. However, attempts to isolate this intermediate were unsuccessful. We attempted to trap the presumed thiolate intermediate via alkylation with 2-bromo-3′-methoxyacetophenone (MP-Br). Treatment of the resulting product with aqueous hydrochloric acid to cleave the methylenoate furnished tetrahydropyridine **11** exclusively, wherein the expected exocyclic olefin had migrated into conjugation with the sulfide. Attempts to photochemically solvolyze the thiophenone via treatment of (7*S*)-**2** with UV irradiation resulted only in nonspecific degradation.

Given the challenges encountered with chemical solvolysis and hydrolysis methods, we turned our attention to biochemical methods for cleaving the thiophenone (Scheme 5). A variety of esterases, proteases, and lipases were screened in parallel with most resulting either in no conversion or in the formation of the oxidized material **10**. The difficulty in opening the thiophenone was most likely due to facile formation of anion **12** in the presence of even weakly basic nucleophiles. As the bioactivation of **2** is mediated by various cytochrome P450 enzymes, it was hypothesized that treatment of **2** with purified liver microsomes might accomplish the conversion of **2** to the active metabolite mixture **3**. Initial studies with rat liver microsomes produced small amounts of the desired products, with human liver microsomes being significantly more efficient. Given the cost of human liver microsomes, we continued screening biological reagents and discovered that less costly induced rat S9 homogenate gave an acceptable product profile (Scheme 5(ii)).

Incubation of 2-oxoclopidogrel (**2**) with rat S9 homogenate followed by preparative SFC and reverse phase HPLC allowed for the isolation of (3*Z*)-**3** clopidogrel metabolites H3 and H4 (along with lesser quantities of (3*E*)-**3** isomers H1 and H2). Isolated metabolites H1−H4 were found to be very unstable in the eluents. On the basis of extensive stability studies, it was discovered that the presence of water was required to prevent dimerization while acetonitrile could slow ester hydrolysis. As such, special handling of the fractions was required during purification including adding water to the SFC fraction receiving reservoir and maintaining the fractions in an ice bath. Additionally, the fractions were partially concentrated via rotary evaporation in an ice bath, followed by lyophilization in an acetonitrile−water solution. Throughout the purification process, avoiding exposure to light was necessary to prevent decomposition. While these methods were able to furnish pure

DOI: 10.1021/acs.joc.5b00632
J. Org. Chem. 2015, 80, 7019−7032

Scheme 5. Enzymatic Hydrolysis of 2-Oxoclopidogrel



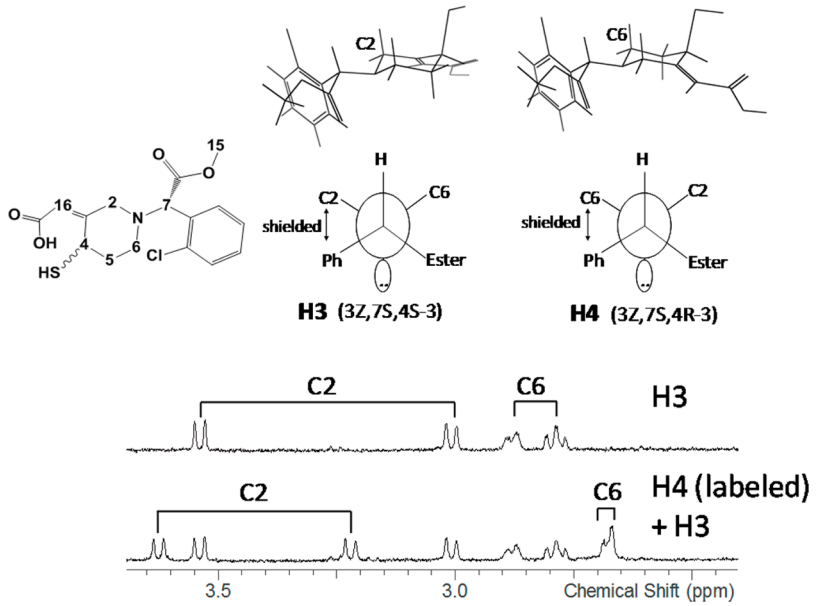


Figure 3. Energy-minimized structures and ^{1}H NMR spectra for stereochemical assignment of 3*Z*-isomers.

H1−H4 in sufficient quantities to enable limited study in biological assays, the instability of the metabolites H1−H4 precluded their use as analytes. Therefore, we chose to target alkylated analogues **13** wherein the thiolate has been alkylated with the strong 3′-methoxyacetophenone chromophore to quantitatively analyze via HPLC with UV detection. Following incubation of (7*S*)-**2** with rat S9 homogenate, the reaction mixture was quenched with 2-bromo-3′-methoxyacetophenone (MP-Br). The resulting product was then resolved via preparative chiral SFC to furnish 4*R*- and 4*S*-isomers of (3*Z*)-**13** along with minor quantities of 4*R*- and 4*S*-isomers of (3*E*)-**13**.

To probe the stereochemical assignment of the 3*Z*-isomers (7*S*-stereocenter defined), molecular mechanics was used to obtain energy-minimized structures for the 4*R*- and 4*S*-isomers (Figure 3). Employing these structures, it was then possible to use the differential anisotropic shielding and deshielding patterns of the C-6 and C-2 methylene groups in each isomer to complete the absolute stereochemistry assignment of the C-4 isomeric metabolites. The C-2 methylene protons of the H3-isomer were upfield compared to H4, consistent with shielding by the phenyl group. In contrast, the C6 methylene protons of H4 were shielded by the phenyl group, as they exhibit upfield chemical shifts compared to H3. As such, the H3 isomer was provisionally assigned as the (3*Z*,4*S*,7*S*)-**3** isomer and H4 was assigned as the (3*Z*,4*R*,7*S*)-**3** isomer. Additionally, efforts to discern the absolute stereochemistry from microsomal incubations were undertaken, and the results were in agreement with the NMR and molecular modeling analysis. Three-minute incubation of 2-oxoclopidogrel ((4*S*,7*S*)-**2**) in human liver microsomes or induced microsomal rat liver S9 produced predominantly isomer H3, while incubations of (4*R*,7*S*)-**2** produced predominatly H4. Given the amount of computational effort required, NMR analysis, and the instability of the compounds being studied, it was decided to continue looking for opportunities to facilitate these stereochemical assignments via chemical synthesis (vide infra).

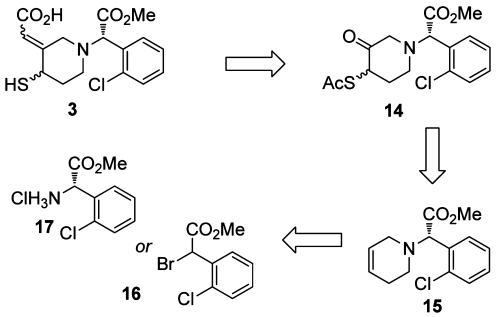
With this method in hand, milligram quantities of analytes were provided to support ongoing clinical evaluation and study of clopidogrel. However, despite our ability to deliver analytical quantities of material, this method had a number of limitations. The cost of reagents and labor-intensive purification limit the scale on which these transformations can be performed. Also, despite being able to provide the 7*S*-isomeric series exclusively,

DOI: 10.1021/acs.joc.5b00632
J. Org. Chem. 2015, 80, 7019−7032

the epimerization of 2-oxoclopidogrel precludes C-4 stereoisomer separation prior to the end of the synthesis and requires SFC methods. As such, a conceptually orthogonal route was considered.

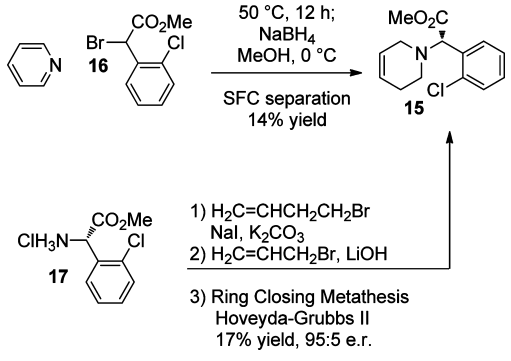
Scalable Stereodivergent Route Development. In evaluating alternative approaches, a number of goals were set forth. First, we wanted to avoid the formation of hydrolytically resistant thiophenone intermediates. Second, we desired a route employing fewer intensive purification steps. Ideally, one synthetic sequence that provided all four metabolites and their corresponding analytes in a single sequence with the facile resolution of the diastereomers would allow for the most efficient method. Based on our previous experience, the unmasking of the C-4 thiol should be conducted as late in the synthetic sequence as possible to prevent oxidation or lactonization with the adjacent enoate. As such, we expected that a mixture of C-3 olefin stereoisomers could be prepared from epimeric ketones **14** via a variety of olefination conditions (Scheme 6). This ketone could be prepared from tetrahydropyridine **15** via a nonfacially selective oxidative addition of the thiocarboxylate to the olefin. Use of **15** would allow the establishment of the C-7 stereochemistry early in the synthetic sequence and minimize late-stage chiral purification. Tetrahydropyridine **15** could be prepared either from halide **16** or amine **17**, both of which are commercially available.

Scheme 6. Stereodivergent Approach Retrosynthetic Analysis



Initial preparation of **15** began with the alkylation of pyridine with alkyl halide **16** (Scheme 7). Careful control of the reaction temperature was key as the resulting pyridinium readily decarboxylates, presumably via the intermediacy of a pyridinium ylide. Careful in situ reduction of the *N*-alkylpyridinium intermediate using methanolic sodium borohydride at 0 °C resulted in the isolation of racemic tetrahydropyridine **15** in 40−50% yield. While the material could be resolved using chiral SFC (14% overall yield of the 7*S*-stereoisomer), this represented an undesirable early-stage bottleneck. Therefore, tetrahydropyridine **15** was prepared in enantioselective fashion from the commercially available arylglycine ester **17** via alkylation of the amine with homoallyl iodide and allyl bromide sequentially. It was necessary to stop each of these alkylation reactions prior to completion to minimize arylglycine epimerization. With resulting diene in hand, tetrahydropyridine **15** was prepared in 95:5 er and 17% overall yield via ring-closing metathesis employing the Hoveyda−Grubbs second-generation ruthenium catalyst.[15] Despite the poor overall yield, both of these protocols were readily scalable and enabled the preparation of multigram quantities of intermediate **15**.

Scheme 7. Tetrahydropyridine 15 Synthesis



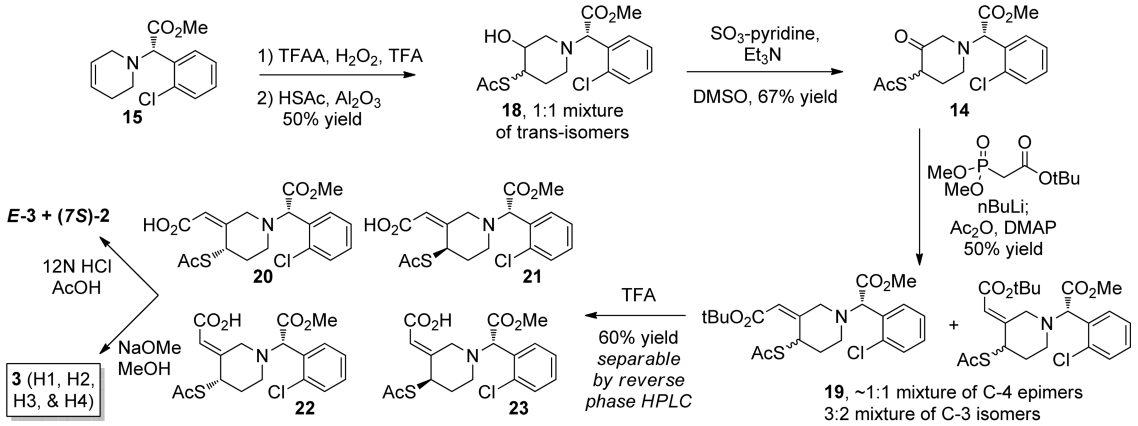
With ample quantities of tetrahydropyridine **15** in hand, the olefin was epoxidized using trifluoroperacetic acid prepared in situ (Scheme 8). It was crucial to use at least 5 equiv of trifluoroacetic acid or the corresponding *N*-oxide was formed in addition to the desired product. The resulting epoxide proved unstable and was concentrated and treated with thioacetic acid and basic alumina to furnish thioacetate **18** as a ca. 1:1 mixture of trans-stereoisomers. The regioselectivity of epoxide ring-opening was exclusive for attack at the methyne distal from the piperidine ring nitrogen, most likely due to destabilization of the S_N2 transition state for attack on the proximal position by the electron-withdrawing piperidine ring nitrogen. Oxidation of alcohols **18** under Parikh−Doering[16] conditions furnished unstable epimeric ketones **14**, which were subsequently subjected to Horner−Wadsworth−Emmons[17] conditions to furnish enoates **19** as a ca. 3:2 mixture of *Z*-/*E*-isomers. In the course of this transformation, some loss of the acetyl group was observed, so the reaction was quenched with acetic anhydride/DMAP to ensure complete reacylation of the C-4 thiols. Subsequent selective unmasking of the enoate with trifluoroacetic acid provided carboxylic acids **20**−**23**, which could be readily separated into the constituent stereoisomers via preparative reverse phase HPLC.

Attempts to deprotect each of the stereoisomeric thioacetates **20**−**23** with HCl resulted in the clean deprotection of the *E*-isomers (**22** and **23**) to furnish the C-4 epimeric (3*E*,7*S*)-**3** isomers; however, the *Z*-isomers (**20** and **21**) cyclized to form 2-oxoclopidogrel (**2**). Fortunately, this observation allowed us to confirm that the C-7 stereocenter does not undergo appreciable epimerization via this synthetic sequence, as this material was identical to a sample of (7*S*)-2-oxoclopidogrel (**2**), with the absolute stereochemistry established via anomalous dispersion X-ray crystallography. Use of 3 equiv of freshly prepared sodium methoxide resulted in the clean conversion of all four stereoisomers **20**−**23** to the corresponding isomeric free thiol metabolites H1−H4 (**3**).

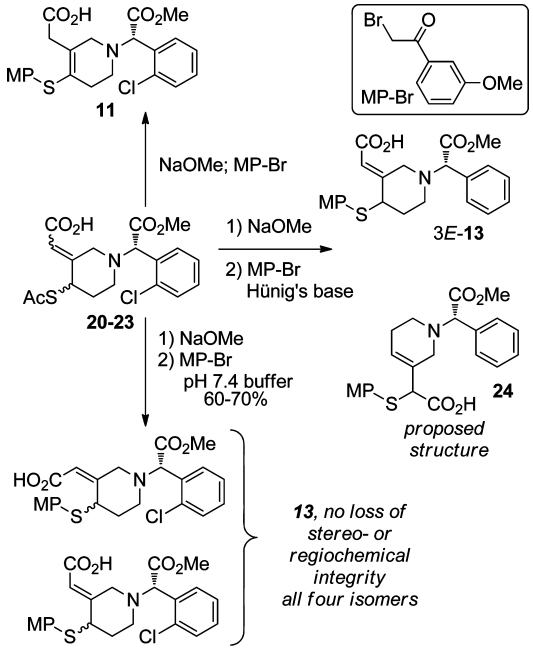
With a scalable synthesis of the four stereoisomers **3** in hand, we turned our attention to the preparation of analytes suitable for clinical evaluation (Scheme 9). Attempts to prepare analytical standards by trapping the crude sodium thiolate with MP-Br produced only the tetrahydropyridine **11**. Treatment of each of the metabolites with Hünig's base and MP-Br cleanly converted the *E*-isomers to the corresponding derivatized analytes **13**; however, the *Z*-isomers produced an isomeric product which we have tentatively assigned as **24** wherein the sulfur position appears to have undergone a 1,3-migration. It is probable that the increased allylic strain present in the *Z*-isomeric metabolites provides the driving force for this

DOI: 10.1021/acs.joc.5b00632
J. Org. Chem. 2015, 80, 7019−7032

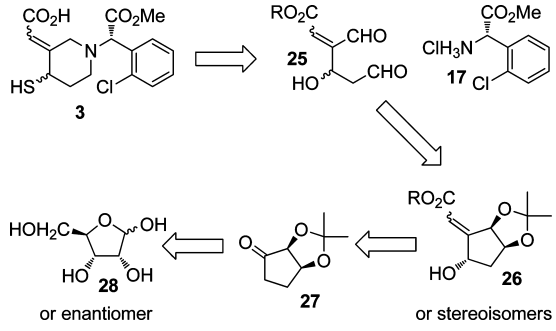
Scheme 8. Clopidogrel Metabolite Synthesis



Scheme 9. Analyte Preparation



Scheme 10. Stereocontrolled Approach Retrosynthetic Analysis



rearrangement. Fortunately, treatment of thioacetates **20**–**23** with sodium methoxide followed by transfer of the solution to pH 7.4 buffer with MP-Br cleanly produced all four of the desired analytes **13** in good yield and with no loss of stereochemical or regiochemical integrity.

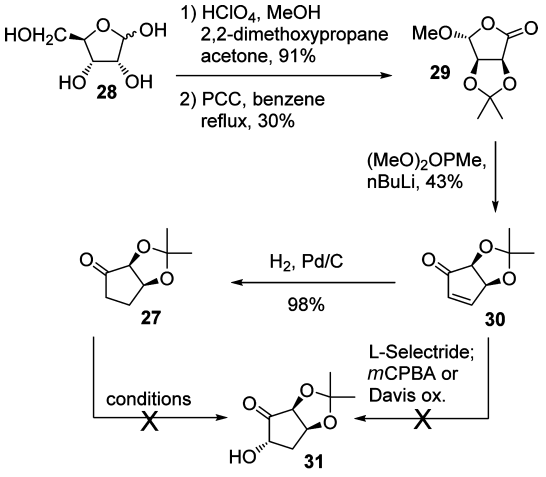
Stereocontrolled Route Development. Despite having access to large quantities of all four isomeric metabolites **3**, the corresponding analytes **13**, and an NMR-based assignment of stereochemistry, rigorous stereochemical assignment of the identity of each isomer was still desirable. As such, our stereocontrolled route to all four active clopidogrel metabolites began by triaging the stereocenters. Previous studies in our laboratories had demonstrated the facile separation of the *E*- and *Z*-isomers, so the problem was reduced to selectively installing both epimers of the C-4 thiol functionality and the 7*S*-configuration of the *o*-chlorophenylglycine moiety (Scheme 10). Our retrosynthetic analysis divided the molecule into the commercially available amine *S*-**17** and dialdehydes **25**. It was envisioned that dialdehydes **25** could be prepared from the known cyclopentanones **27** where the stereochemistry of the cyclic acetonide could be used to control the stereochemistry at the eventual C-4 position. Cyclopentanones **27** are readily available from either D- or L-ribose (**28**) depending on the desired C-4 stereochemistry of the final product.[18]

Cyclopentanone **27** was prepared from D-ribose (**28**), starting with concomitant protection of the C-2/C-3 diol as the acetonide and formation of the methyl glycoside (Scheme 11). This reaction was easily performed on large scale (up to 20 g), and oxidative cleavage with PCC furnished lactone **29**. Treatment of **29** with lithiated dimethyl methylphosphonate produced enone **30** via nucleophilic ring-opening of the lactone followed by an intramolecular Horner–Wadsworth–Emmons reaction.[18] Attempts to effect a one-pot, tandem conjugate reduction/oxidation of enone **30** failed to produce any of the desired α-hydroxy cyclopentanone **31**. Alternatively, hydrogenation of enone **30** using Pd/C furnished cyclopentanone **27** in 98% yield. All attempts to oxidize the ketone provided complex reaction mixtures.

Surmising that the difficulties isolating the desired product probably reflected poor intrinsic stability of **31**, we chose to delay installation of the nascent C-4 stereocenter and turned our attention to the C-3 enone (Scheme 12). Horner–Wadsworth–Emmons olefination of ketone **27** yielded enoates **32** as an inseparable 1:1 mixture of *E*- and *Z*-isomers. Treatment of this mixture with selenium dioxide produced allylic alcohols **34** and **35** which were readily separable by silica gel chromatography, thus providing stereodefined access to both the C-3 enoate and a progenitor to the C-4 thiol. Interestingly, an alternative hydroxylation protocol proceeding via the intermediacy of **33** only yielded the *Z*-isomeric enoate although the reasons for this level selectivity are not clear at this time. Subsequently, the acetonides of both **34** and **35** were

DOI: 10.1021/acs.joc.5b00632
J. Org. Chem. 2015, 80, 7019–7032

Scheme 11. Establishment of Latent C-4 Stereochemistry

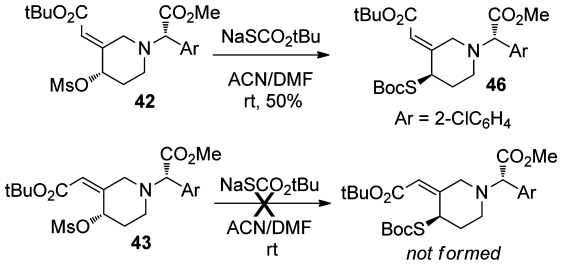


Entry	Reagents	Outcomes
1	LDA; MoOPh	complex mixture
2	LDA; $(TMSO)_2$	Baeyer-Villiger
3	NaHMDS or LDA Davis oxaziridine	complex mixture
4	TMSOTf, TEA; *m*CPBA, DMDO or OsO_4	complex mixture
5	TMSOTf, TEA; DMDO; Ac_2O	trace product observed

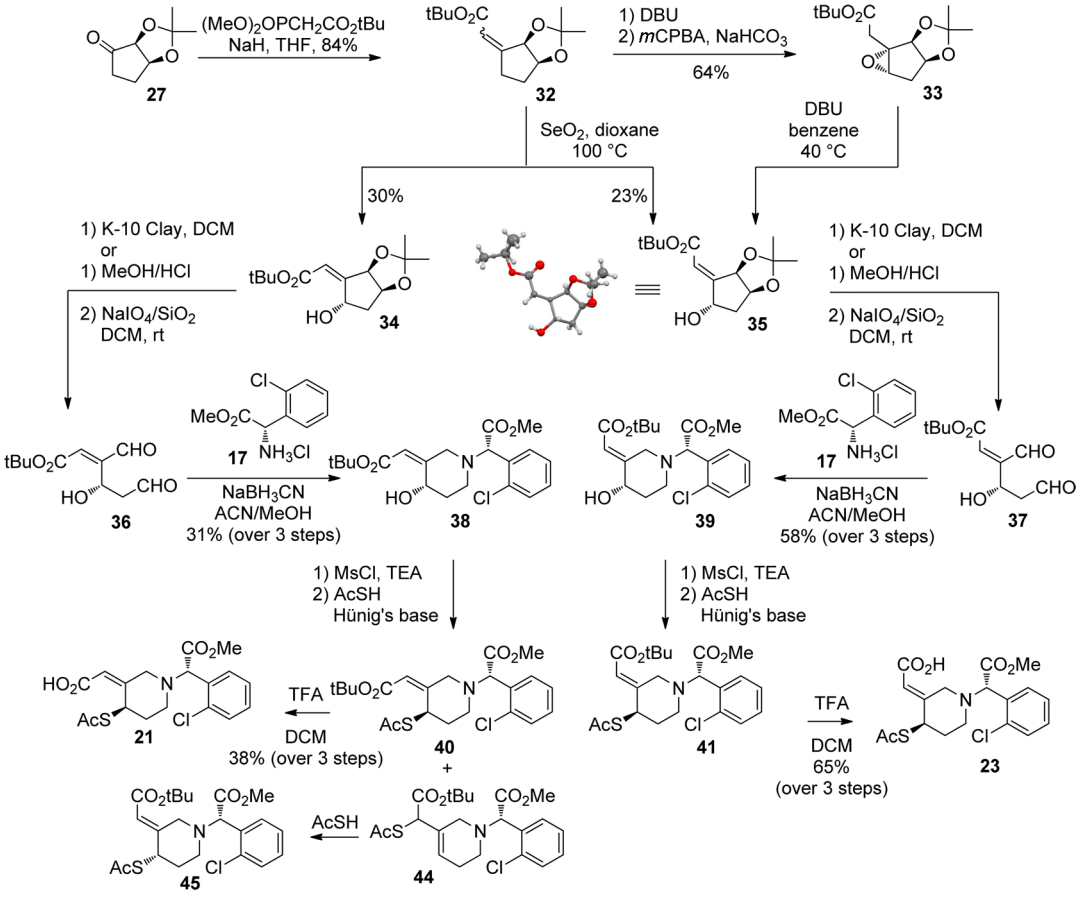
deprotected with K-10 Montmorillionite clay (or HCl in MeOH) and oxidatively cleaved with silica-supported $NaIO_4$[19] to provide dialdehydes **36** and **37**.

With dialdehydes **36** and **37** in hand, *E*- and *Z*-isomeric piperidines could be prepared via tandem reductive aminations with commercially available (7*S*)-**17** and $NaBH_3CN$. Each of these isomers could be converted to the corresponding mesylate using mesyl chloride and triethylamine. No formation of chloride intermediates were detected, nor was there any evidence of epimerization of the C-4 position. All that remained was the displacement of the mesylate with an acceptable thiol precursor. Given our previous success with acid mediated removal of *tert*-butyl esters and solvolysis of thioacetates, $NaSCO_2tBu$[20] and thioacetic acid were selected as potential thiol sources. In the case of the *tert*-butyl thiocarbonate, the displacement and deprotection worked well on the *E*-isomeric mesylate **42**; however, the *Z*-isomer **43** failed to yield any displaced product (Scheme 13). Conversely, displacement of

Scheme 13. Mesylate Thiol Displacement Studies



Scheme 12. Stereocontrolled Preparation of Clopidogrel Metabolite Thioacetate Intermediates

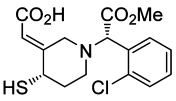
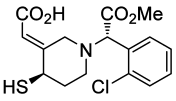
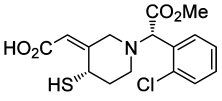
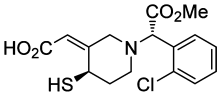


DOI: 10.1021/acs.joc.5b00632
J. Org. Chem. 2015, 80, 7019−7032

the mesylate on *E*- and *Z*-isomers with thioacetic acid produced the thioacetates **40** and **41**, respectively, which could in turn be deprotected to thioacetates **21** and **23** and from there to metabolites **3** according to the conditions previously described (Scheme 8). Interestingly, while the *E*-isomer **41** was formed cleanly, the *Z*-isomer **40** was contaminated with S_N2' product **44** and a minor quantity of the C-4 epimer **45**. Furthermore, treatment of the S_N2' **44** with thioacetic acid produced exclusively **45**, suggesting the mechanism of formation of this byproduct.

The C-4 epimers of thioacetates **21** and **23** (**20** and **22**, respectively) were prepared from L-ribose according to the same methods and elaborated to the corresponding isomers of **3**, completing the stereochemical assignment of all the active metabolites. Intersection of the previously described route had the added benefit of allowing flexibility in terms of which route to employ, as now all stereoisomers from both routes could be assigned by correlation of HPLC retention times. By chemical correlation of the synthetic material with the in vivo samples, we were able to conclusively assign the stereochemistry of the previously delineated active clopidogrel stereoisomers H1−H4 (Table 1).

Table 1. Active Clopidogrel Metabolite Stereochemical Assignment

Metabolite ID	Stereochemistry
H1	 (3E,4S,7S)-**3**
H2	 (3E,4R,7S)-**3**
H3	 (3Z,4S,7S)-**3**
H4	 (3Z,4R,7S)-**3**

CONCLUSION

In summary, we have demonstrated three conceptually orthogonal methods for the preparation of all bioactive metabolites of clopidogrel. The complete unambiguous assignment of stereochemical configuration for each metabolite was established. Moreover, in addition to providing substantial quantities of material for improved understanding of clopidogrel in vivo activity, the biomimetic route sheds additional light on the mechanism of hydrolytic thiophenone bioactivation in the liver.

EXPERIMENTAL SECTION

General Information: Organolithium reagents were titrated with *N*-*o*-tolylpivalamide in THF. Methanolic NaOH solutions were freshly prepared from pellet NaOH prior to use. Molecular sieves were flame-dried under vacuum. All other reagents were used as received from the manufacturer. Analytical thin layer chromatography (TLC) was accomplished using EMD TLC silica gel 60 F245 plates and visualized with the aid of UV light. Analytical HPLC was accomplished using a Luna 3 μm C18 2.0 × 30 mm column, 10−90% acetonitrile (ACN) in 0.1% TFA/water. All HRMS data were collected via a time-of-flight mass analyzer unless otherwise specified. Flash chromatography was accomplished according to the Still procedure using Teledyne Isco RediSep normal phase disposable columns. All reactions were performed under an atmosphere of argon.

Molecular Modeling and Computations: Possible enantiomers of *Z*-**3** were evaluated as follows. Distinct conformers were generated via the MacroModel 9.5[21] conformational analysis module in Maestro 8.0,[22] using the OPLS_2005 force field and a $CHCl_3$ solution environment. These conformers were next subjected to a quantum-chemical protocol, which involved total geometry optimization at the B3-LYP/6-31+G(d,p)[23,24] level of Kohn−Sham density functional theory (DFT),[25] followed by calculation of a final single-point energy at RI-MP2/6-31+G(d,p).[26] All quantum-chemical calculations used the Q-Chem 3.1 software.[27] As a last step, Boltzmann weights were calculated for all conformers from the RI-MP2 energies relative to the lowest-energy conformer.

5-Trityl-5,6,7,7a-tetrahydrothieno[3,2-*c*]pyridin-2(4*H*)-one 6. A solution of 2-(thiophen-2-yl)ethanamine (100 g, 786 mmol) in toluene (500 mL) was charged in a 1 L flask equipped with a Dean−Stark trap. To the solution was added paraformaldehyde (26.0 g, 865 mmol), and the reaction mixture was heated to reflux for 1.5 h. The reaction mixture was cooled to room temperature, and the solution was added through an addition funnel into a 3 L three-necked flask equipped with mechanical stirrer and 4 M hydrogen chloride in dioxane (230 mL, 920 mmol) under N_2. The addition was finished in 25 min, and the temperature reached 65 °C. The mixture was stirred vigorously at 60 °C for 30 min and then cooled to room temperature. The solid was collected as a light yellow HCl salt by filtration, washed with ether, and oven-dried to give 4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine, HCl salt (135 g, 770 mmol, 98% yield). MS-ESI $[M + H]^+$ 140.0 (139.1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.79 (br s, 2H), 7.45 (d, J = 5.1 Hz, 1H), 7.07−6.78 (m, 1H), 4.37−3.93 (m, 2H), 3.42−3.28 (m, 2H), 3.05 (t, J = 6.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 132.2, 128.79, 125.9, 125.1, 42.5, 41.2, 21.9. To a solution of 4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine, HCl salt (55.5 g, 316 mmol) in CH_2Cl_2 (1 L) were added triethylamine (63.9 g, 631 mmol) and chlorotriphenylmethane (80. g, 290 mmol), the mixture was stirred at room temperature for 2 h, and then water was added. The layers were separated, and the aqueous phase was extracted with CH_2Cl_2 (500 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give 5-trityl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (109 g, 287 mmol, 100% yield) which was used in the next step without further purification. A solution of *n*-butyllithium (149 mL, 371 mmol, 2.5 M in hexanes) was added to a solution of 5-trityl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (109 g, 286 mmol) in THF (1500 mL) under argon at room temperature. After stirring for 1 h, trimethyl borate (35.0 mL, 314 mmol) was added over 10 min. The resulting mixture was stirred at room temperature for 1 h. An aqueous solution of hydrogen peroxide (35.3 mL, 571 mmol) was added to the reaction solution over 10 min. The mixture was stirred for 1 h and diluted with EtOAc, and the layers were separated. The aqueous phase was extracted with EtOAc (4 × 500 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give the crude product which was purified by recrystallization from MeOH to give **6** (100 g, 252 mmol, 88% yield) as an off-white solid. IR (neat, cm^{-1}) 3550, 3474, 3413, 3054, 2921, 2813, 1682, 1645, 1488, 1447, 1095, 982, 902, 753. HRMS calcd for $C_{26}H_{24}NOS$ $[(M + H)^+]$ 398.1579, found 398.1566. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.51−7.27 (m, 12H), 7.26−7.15 (m, 3H), 6.23 (s, 1H), 4.39 (td, J = 6.0, 5.0 Hz, 1H), 4.04 (dd, J = 11.9, 2.4 Hz, 1H), 3.17 (dd, J = 12.0, 2.7 Hz, 1H), 2.47−2.38 (m, 1H), 2.12 (d, J = 11.6 Hz, 1H), 1.96 (qd, J = 12.0, 3.6 Hz, 1H), 1.70−1.56 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 199.0, 171.9, 129.1, 128.4, 126.8, 125.4, 77.5, 51.5, 50.5, 47.6, 35.2.

DOI: 10.1021/acs.joc.5b00632
J. Org. Chem. 2015, 80, 7019−7032

5,6,7,7a-Tetrahydrothieno[3,2-*c*]pyridin-2(4*H*)-one HCl Salt 8. To a 2 L flask were added 5-trityl-5,6,7,7a-tetrahydrothieno[3,2-*c*]pyridin-2(4*H*)-one (30. g, 75 mmol) and 2.5 M hydrogen chloride in EtOH (302 mL, 755 mmol). The mixture was stirred at 45 °C to give a suspension first and then a clear orange solution. The reaction was stirred overnight. The solid precipitated from the EtOH solution was collected by filtration and discarded. The solution was concentrated to dryness. The residue was stirred in ethyl ether (3 × 300 mL), and the ether layer was discarded to remove chlorotriphenylmethane. The residue was then stirred in isopropanol (IPA) (50 mL). The product was collected by filtration as an off-white solid, washed with ether and IPA, and concentrated to give **8** (11 g, 57 mmol, 76% yield). IR (neat, cm^{-1}) 3414, 3064, 2951, 2843, 2805, 1742, 1682, 1591, 1473, 1435, 1203, 1169, 1094, 1041, 1006, 857, 755. HRMS calcd for $C_7H_{10}NOS$ $[(M + H)^+]$ 156.0483, found 156.0475; ^1H NMR (400 MHz, MeOD) δ ppm 6.44 (s, 1H), 4.68 (dd, J = 12.6, 5.5 Hz, 1H), 4.47 (dd, J = 13.9, 1.4 Hz, 1H), 4.04 (d, J = 13.8 Hz, 1H), 4.04 (d, J = 13.8 Hz, 1 H), 3.55−3.64 (m, 1H), 3.32−3.43 (m, 1H), 2.65−2.76 (m, 1H), 1.81−1.96 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 197.8, 164.6, 129.5, 49.2, 44.0, 42.7, 31.1.

Methyl 2-(2-Chlorophenyl)-2-(2-oxo-7,7a-dihydrothieno-[3,2-*c*]pyridin-5(2*H*,4*H*,6*H*)-yl)acetate 2. To a solution of 5,6,7,7a-tetrahydrothieno[3,2-*c*]pyridin-2(4*H*)-one hydrochloride (11.5 g, 60.0 mmol) in DMF (100 mL) were added $KHCO_3$ (12 g, 120 mmol) and sodium iodide (8.99 g, 60.0 mmol). The suspension was stirred at 55 °C for 3 min to give a dark solution. Then methyl 2-chloro-2-(2-chlorophenyl)acetate (13.1 g, 60.0 mmol) was added. The solution was heated at 55 °C, and the reaction was complete after 10 min. The resulting mixture was poured into water (300 mL), extracted with EtOAc (300 mL), and washed with brine three times. The organic layer was dried over Na_2SO_4 and then concentrated to furnish a black viscous oil (23.5 g). The product was purified by flash chromatography using 10−20% MeOH/CH_2Cl_2 as eluent, yielding methyl 2-(2-chlorophenyl)-2-(2-oxo-7,7a-dihydrothieno[3,2-*c*]pyridin-5(2*H*,4*H*,6*H*)-yl)acetate as a dark viscous oil (9.0 g, 27 mmol, 44% yield). Separation of stereoisomers was effected using ChiralCel OJ-H 25 × 3 cm ID, 5 μm; 130 mL/min, 40 °C, 80% CO_2−20% MeOH. t_R [(4*R*,7*S*-**2**)] = 3.5 min; t_R [(4*S*,7*R*-**2**)] = 5.5 min. IR (neat, cm^{-1}) 3391, 2936, 2789, 2458, 1722, 1682, 1592, 1445, 1197, 1092, 755. HRMS calcd for $C_{16}H_{17}ClNO_3S$ $[(M + H)^+]$ 338.0618, found 338.0608. ^1H NMR (600 MHz, $CDCl_3$) δ 7.52 (m, 1H), 7.44 (m, 1H), 7.32 (m, 2H), 6.01 (s, 1H), 4.89 (s, 1H), 4.17 (dd, J = 11.9, 5.7 Hz, 1H), 3.84 (dd, J = 12.6, 1.5 Hz, 1H), 3.73 (s, 3H), 3.17 (d, J = 12.6 Hz, 1H), 3.11 (m, 1H), 2.67 (td, J = 12.2, 2.1 Hz, 1H), 2.41−2.35 (m, 1H), 1.88 (qd, J = 12.6, 4.0 Hz, 1H). t_R [(4*S*,7*S*-**2**)] = 4.5 min; t_R [(4*R*,7*R*-**2**)] = 3.5 min. ^1H NMR (600 MHz, $CDCl_3$) δ 7.53 (m, 1H), 7.44 (m, 1H), 7.31 (m, 2H), 6.03 (s, 1H), 4.91 (s, 1H), 4.17 (dd, J = 12.3, 5.6 Hz, 1H), 3.93 (d, J = 12.3 Hz, 1H), 3.74 (s, 3H), 3.25 (d, J = 12.3 Hz, 1H), 3.05−3.00 (m, 1H), 2.61 (t, J = 11.4 Hz, 1H), 2.39−2.33 (m, 1H), 1.88 (qd, J = 12.5, 3.8 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 198.7, 170.8, 167.2, 134.8, 132.8, 130.1, 129.8, 129.7, 127.2, 126.8, 67.3, 52.3, 51.7, 51.1, 49.6, 33.8

Racemic Synthesis of Methyl 2-(2-Chlorophenyl)-2-(5,6-dihydropyridin-1(2*H*)-yl)acetate 15. To a solution of methyl 2-bromo-2-(2-chlorophenyl)acetate (13.1 g, 49.6 mmol) in acetonitrile (25 mL) was added pyridine (8 mL, 100 mmol), and the mixture was heated to 50 °C for 16 h. The solvent was concentrated, and crude 1-(1-(2-chlorophenyl)-2-methoxy-2-oxoethyl)pyridinium bromide was redissolved in MeOH (330 mL) and cooled to −20 °C. To this solution was added sodium borohydride (3.8 g, 99 mmol) portionwise. The reaction was allowed to warm to room temperature, concentrated, and partitioned between EtOAc and water. Then the organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography using hexanes/EtOAc as eluant, yielding pure **15** as a pale yellow oil (11.5 g, 84%).

Enantioselective Synthesis of (*S*)-Methyl 2-(2-Chlorophenyl)-2-(5,6-dihydropyridin-1(2*H*)-yl)acetate 15. To a slurry of (*S*)-methyl 2-amino-2-(2-chlorophenyl)acetate hydrochloride (0.050 g, 0.21 mmol), potassium carbonate (0.126 g, 0.911 mmol), and sodium iodide (0.105 g, 0.699 mmol) in DMF (0.25 mL) was added 4-bromobut-1-ene (0.020 mL, 0.19 mmol), and the mixture was stirred at ambient for 18 h. The reaction was partitioned between water and ether, and the aqueous phase was washed 2× with ether. The combined organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography using 15% CH_2Cl_2/ether as eluant, yielding (*S*)-methyl 2-(but-3-enylamino)-2-(2-chlorophenyl)acetate as a clear oil of sufficient purity for subsequent steps (0.0287 g, 53%). MS-ESI $[M + H]^+$ 254.3 (253.1). To a suspension of (*S*)-methyl 2-(but-3-enylamino)-2-(2-chlorophenyl)acetate (0.0287 g, 0.113 mmol) and molecular sieves 4 Å (0.050 g) in DMF (0.28 mL) were added in quick succession lithium hydroxide hydrate (0.0050 g, 0.10 mmol) and 3-bromoprop-1-ene (10 μL, 0.1 mmol), and the mixture was stirred for 18 h. The reaction was partitioned between water and ether, and the aqueous phase was washed with ether. The combined organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography using hexanes/EtOAc yielding (*S*)-methyl 2-(allyl(but-3-enyl)amino)-2-(2-chlorophenyl)acetate as a clear oil of sufficient purity for subsequent steps (0.014 g, 41%). MS-ESI $[M + H]^+$ 294.4 (293.1). To a solution of (*S*)-methyl 2-(allyl(but-3-enyl)amino)-2-(2-chlorophenyl)acetate (0.011 g, 0.037 mmol) in CH_2Cl_2 (3.7 mL) were added TsOH (7 mg, 0.03 mmol) and Hoveyda−Grubbs catalyst[18] (5.0 mg, 7.0 μmol), and the mixture was refluxed for 100 min. The reaction was concentrated and the residue purified by HPLC (Luna 5 μm C18 30 × 250 mm, 10−90% ACN in TFA buffered water), and free-based with sodium bicarbonate to furnish pure **15** as a clear oil (0.008 g, 80%, 90.6% ee). SFC (Chiral OJ-H 250 × 4.6 mm ID, 5 μm, 95/5 CO_2/MeOH, 100 bar, 2.0 mL/min) t_R = 3.4 min [(*S*)-isomer], t_R = 3.2 min [(*R*)-isomer]; HRMS calcd for $C_{14}H_{17}ClNO_2$ $[(M + H)^+]$ 266.0948, found 266.0933 (ion trap); ^1H NMR (500 MHz, $CDCl_3$) δ ppm 7.70 (dd, J = 7.7, 1.9 Hz, 1H), 7.39 (dd, J = 7.8, 1.5 Hz, 1H), 7.31−7.22 (m, 2H), 5.81−5.73 (m, 1H), 5.66−5.59 (m, 1H), 4.77 (s, 1H), 3.71 (s, 3H), 3.23−3.13 (m, 1H), 3.07−2.98 (m, 1H), 2.69−2.58 (m, 2H), 2.18 (qt, J = 5.6, 2.9 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 171.4, 134.7, 133.9, 130.0, 129.6, 129.2, 127.1, 125.2, 124.9, 68.5, 52.0, 50.4, 47.4, 26.1.

(*E*)-2-((*S*)-4-(Acetylthio)-1-((*S*)-1-(2-chlorophenyl)-2-methoxy-2-oxoethyl)piperidin-3-ylidene)acetic Acid 22 with (*E*)-2-((*R*)-4-(Acetylthio)-1-((*S*)-1-(2-chlorophenyl)-2-methoxy-2-oxoethyl)piperidin-3-ylidene)acetic Acid 23 and (*Z*)-2-((*S*)-4-(acetylthio)-1-((*S*)-1-(2-chlorophenyl)-2-methoxy-2-oxoethyl)-piperidin-3-ylidene)acetic Acid 20 and (*Z*)-2-((*R*)-4-(acetylthio)-1-((*S*)-1-(2-chlorophenyl)-2-methoxy-2-oxoethyl)piperidin-3-ylidene)acetic Acid 21. To a solution of hydrogen peroxide (1.2 mL, 19 mmol) in CH_2Cl_2 (90 mL) was added TFAA (5.3 mL, 38 mmol) at 0 °C. Solution was stirred for 90 min and then treated with a solution of methyl 2-(2-chlorophenyl)-2-(5,6-dihydropyridin-1(2*H*)-yl)acetate (2.00 g, 7.50 mmol) and TFA (2.9 mL, 38 mmol) in CH_2Cl_2 (12 mL). The reaction was complete after 10 min. The solution was treated with a solution of Na_2SO_3 in water and stirred 10 min at room temperature. The mixture was neutralized with 1 M Na_2CO_3 (ca. 100 mL) solution and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated and the residue used as methyl 2-(7-oxa-3-azabicyclo[4.1.0]heptan-3-yl)-2-(2-chlorophenyl)acetate without further manipulation. To a slurry of alumina (25 g, 7.5 mmol) in ethyl ether (75 mL) was added thioacetic acid (2.70 mL, 37.7 mmol). After 10 min, a solution of methyl 2-(7-oxa-3-azabicyclo[4.1.0]heptan-3-yl)-2-(2-chlorophenyl)acetate (2.12 g, 7.53 mmol) in ether (20 mL) was added and the reaction stirred 16 h. The reaction was filtered and washed with $NaHCO_3$. The solution was concentrated and the residue filtered through a plug of silica gel using hexanes/EtOAc as eluant, to furnish methyl 2-(4-(acetylthio)-3-hydroxypiperidin-1-yl)-2-(2-chlorophenyl)acetate (1.54 g, 57% yield). The intermediate was sufficiently pure for subsequent steps, and no further purification was performed. To a solution of methyl 2-(4-(acetylthio)-3-hydroxypiperidin-1-yl)-2-(2-chlorophenyl)acetate (1.07 g, 2.99 mmol) in DMSO (15 mL) was added Et_3N (2.1 mL, 15 mmol), and the solution was cooled to ca. 0−5 °C. To this solution was added a solution of sulfur trioxide pyridine complex (2.4 g, 15 mmol) in DMSO (15 mL), and

DOI: 10.1021/acs.joc.5b00632
J. Org. Chem. 2015, 80, 7019−7032

the reaction was allowed to warm to room temperature and stirred for 4 h. The reaction was cooled to 0 °C, quenched with water, and partitioned and extracted with EtOAc. The organic layer was concentrated and the residue filtered through a plug of silica gel using hexanes/EtOAc as eluant to furnish methyl 2-(4-(acetylthio)-3-oxopiperidin-1-yl)-2-(2-chlorophenyl)acetate (0.49 g, 1.4 mmol, 46% yield). The intermediate was sufficiently pure for subsequent steps, and no further purification was performed. To a solution of *tert*-butyl 2-(dimethoxyphosphoryl)acetate (0.33 mL, 1.65 mmol) in THF (6.9 mL) at −78 °C was added *n*-BuLi (0.66 mL, 1.7 mmol). The solution was stirred 20 min and then treated with methyl 2-(4-(acetylthio)-3-oxopiperidin-1-yl)-2-(2-chlorophenyl)acetate (0.49 g, 1.4 mmol) as a solution in THF (2.3 mL). The resulting solution was allowed to warm slowly to room temperature. The solution was then treated with 0.5 mL of Ac_2O and ca. 10 mg of DMAP and stirred 10 min. The resulting solution was partitioned between EtOAc and sat. aq bicarbonate. The organic layer was concentrated and the residue filtered through a plug of silica gel using hexanes/EtOAc as eluant to furnish (*E*)-methyl 2-(4-(acetylthio)-3-(2-*tert*-butoxy-2-oxoethylidene)piperidin-1-yl)-2-(2-chlorophenyl)acetate with (*Z*)-methyl 2-(4-(acetylthio)-3-(2-*tert*-butoxy-2-oxoethylidene)piperidin-1-yl)-2-(2-chlorophenyl)acetate (1:1) (0.31 g, 0.67 mmol, 49% yield). The intermediate was sufficiently pure for subsequent steps, and no further purification was performed. To a solution of (*E*)-methyl 2-(4-(acetylthio)-3-(2-*tert*-butoxy-2-oxoethylidene)piperidin-1-yl)-2-(2-chlorophenyl)acetate with (*Z*)-methyl 2-(4-(acetylthio)-3-(2-*tert*-butoxy-2-oxoethylidene)-piperidin-1-yl)-2-(2-chlorophenyl)acetate (1:1 ratio) (0.31 g, 0.67 mmol) in CH_2Cl_2 (13 mL) was added TFA (1.5 mL, 20 mmol), and the mixture was stirred for 2 h. The solution was concentrated and purified by HPLC (Luna 5u C18 30 × 250 mm, 10−50% ACN in TFA buffered water) to furnish four fractions.

Peak 1: (*E*)-2-((*S*)-4-(Acetylthio)-1-((*S*)-1-(2-chlorophenyl)-2-methoxy-2-oxoethyl)piperidin-3-ylidene)acetic Acid Trifluoroacetate 22. (0.065 g, 0.13 mmol, 19% yield), IR (neat, cm^{-1}) 1712, C═O; 1687, C═O; 1666, C═O; HRMS (ESI+) *m*/*z* Calcd for $C_{18}H_{20}ClNO_5S$ [M + H^+] 398.0823, found 398.0820 (ion trap); ^1H NMR (400 MHz, $CDCl_3$) δ ppm 8.77 (br s, 3H), 7.75−7.64 (m, 1H), 7.57−7.50 (m, 1H), 7.48−7.40 (m, 2H), 6.36 (s, 1H), 5.52 (s, 1H), 5.14 (d, *J* = 13.7 Hz, 1H), 4.47 (t, *J* = 4.1 Hz, 1H), 4.13 (d, *J* = 13.7 Hz, 1H), 3.78 (s, 3H), 3.53−3.42 (m, 1H), 3.23 (d, *J* = 13.2 Hz, 1H), 2.62−2.48 (m, 1H), 2.36 (s, 3H), 2.03 (dd, *J* = 15.4, 3.8 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 192.4, 167.4, 167.0, 144.2, 135.0, 132.1, 130.8, 128.6, 126.8, 123.5, 64.3, 53.7, 47.9, 47.5, 43.9, 30.8, 28.2.

Peak 2: (*E*)-2-((*R*)-4-(Acetylthio)-1-((*S*)-1-(2-chlorophenyl)-2-methoxy-2-oxoethyl)piperidin-3-ylidene)acetic Acid Trifluoroacetate 23. (0.042 g, 0.081 mmol, 12% yield), IR (neat, cm^{-1}) 1749, C═O; 1697, C═O; 1658, C═O; HRMS (ESI+) *m*/*z* Calcd for $C_{18}H_{20}ClNO_5S$ [M + H^+] 398.0823, found 398.0824 (ion trap); ^1H NMR (400 MHz, $CDCl_3$) δ ppm 8.40 (br s, 3H), 7.71−7.64 (m, 1H), 7.54−7.50 (m, 1H), 7.48−7.38 (m, 2H), 6.30 (s, 1H), 5.49 (s, 1H), 4.81 (d, *J* = 13.7 Hz, 1H), 4.41 (t, *J* = 5.2 Hz, 1H), 4.29 (d, *J* = 13.7 Hz, 1H), 3.80 (s, 3H), 3.55−3.45 (m, 1H), 3.21−3.09 (m, 1H), 2.51 (td, *J* = 9.9, 4.4 Hz, 1H), 2.37 (s, 3H), 2.14−2.02 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 192.3, 167.0, 144.4, 135.3, 132.1, 130.9, 130.8, 128.4, 127.0, 122.6, 65.4, 53.8, 49.2, 48.3, 43.9, 30.8, 28.8.

Peak 3: (*Z*)-2-((*S*)-4-(Acetylthio)-1-((*S*)-1-(2-chlorophenyl)-2-methoxy-2-oxoethyl)piperidin-3-ylidene)acetic Acid Trifluoroacetate 20. (0.070 g, 0.14 mmol, 20% yield), IR (neat, cm^{-1}) 1751, C═O; 1697, C═O; 1658, C═O; HRMS (ESI+) *m*/*z* Calcd for $C_{18}H_{20}ClNO_5S$ [M + H^+] 398.0823, found 398.0816 (ion trap); ^1H NMR (400 MHz, $CDCl_3$) δ ppm 9.27 (br s, 3H), 7.62 (d, *J* = 7.1 Hz, 1H), 7.55−7.50 (m, 1H), 7.49−7.39 (m, 2H), 5.95 (s, 1H), 5.81 (br s, 1H), 5.48 (s, 1H), 3.89−3.69 (m, 5H), 3.41 (d, *J* = 11.5 Hz, 1H), 3.32−3.22 (m, 1H), 2.61−2.48 (m, 1H), 2.32 (s, 3H), 2.00 (d, *J* = 13.2 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 192.8, 168.0, 167.4, 146.8, 135.2, 131.6, 131.1, 130.7, 128.1, 121.3, 65.9, 54.4, 53.4, 47.3, 38.2, 30.7, 28.7.

Peak 4: (*Z*)-2-((*R*)-4-(Acetylthio)-1-((*S*)-1-(2-chlorophenyl)-2-methoxy-2-oxoethyl)piperidin-3-ylidene)acetic Acid Trifluoroacetate 21. (0.042 g, 0.083 mmol, 12% yield), IR (neat, cm^{-1}) 1747, C═O; 1697, C═O; 1658, C═O; HRMS (ESI+) *m*/*z* Calcd for $C_{18}H_{20}ClNO_5S$ [M + H^+] 398.0823, found 398.0821 (ion trap); ^1H NMR (400 MHz, $CDCl_3$) δ ppm 8.29 (br s, 3H), 7.62 (dd, *J* = 7.1, 2.2 Hz, 1H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.47−7.38 (m, 2H), 5.87 (s, 1H), 5.80 (br s, 1H), 5.44 (s, 1H), 3.78 (s, 4H), 3.64 (d, *J* = 13.2 Hz, 1H), 3.50 (d, *J* = 12.1 Hz, 1H), 3.21−3.09 (m, 1H), 2.60−2.46 (m, 1H), 2.32 (s, 3H), 2.07−1.96 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 192.8, 168.0, 167.5, 147.1, 135.4, 131.5, 130.9, 130.7, 128.0, 120.9, 65.9, 55.2, 53.3, 46.5, 38.3, 30.7, 28.7.

*Representative Procedure for the Formation of Clopidogrel Metabolites. (E)-2-((S)-1-((S)-1-(2-Chlorophenyl)-2-methoxy-2-oxoethyl)-4-mercaptopiperidin-3-ylidene)acetic Acid Trifluoroacetate (3E,4S,7S)-**3** (H1).* To a solution of (*E*)-2-((*S*)-4-(acetylthio)-1-((*S*)-1-(2-chlorophenyl)-2-methoxy-2-oxoethyl)piperidin-3-ylidene)acetic acid trifluoroacetate **22** (0.050 g, 0.098 mmol) in MeOH (4.9 mL) was added NaOH (0.293 mL, 0.29 mmol, 1 M solution in MeOH). After 60 min, the solution was treated with ca. 0.3 mL of 90/10 water/ACN/0.1% TFA and concentrated. The residue was purified by reverse phase HPLC (Luna 5u C18 30 × 250 mm, 10−90% ACN in TFA buffered water) to furnish (*E*)-2-((*S*)-1-((*S*)-1-(2-chlorophenyl)-2-methoxy-2-oxoethyl)-4-mercaptopiperidin-3-ylidene)acetic acid trifluoroacetate (3 *E*,4*S*,7*S*)-**3** (0.035 g, 0.074 mmol, 76%). IR (CD_3CN, cm^{-1}) 2565, S−H; 1791, C═O; 1752, C═O; 1726, C═O; 1198, C−O; 1166, C−O; HRMS (ESI+) *m*/*z* Calcd for $C_{16}H_{19}ClNO_4S$ [M + H^+] 356.0723, found 356.0720 (ion trap); ^1H NMR (500 MHz, CD_3CN) δ 7.65 (dd, *J* = 3.4, 1.5 Hz, 1H), 7.64 (dd, *J* = 3.2, 1.5 Hz, 1H), 7.59 (td, *J* = 7.8, 1.8 Hz, 1H), 7.55−7.50 (m, 1H), 6.28 (s, 1H), 5.56 (s, 1H), 4.75 (d, *J* = 13.8 Hz, 1H), 4.46 (d, *J* = 13.8 Hz, 1H), 4.08 (t, *J* = 4.8 Hz, 1H), 3.80 (s, 3H), 3.71−3.57 (m, 1H), 3.40 (d, *J* = 12.9 Hz, 1H), 2.49 (ddt, *J* = 15.4, 10.8, 4.2 Hz, 1H), 2.07−2.00 (m, 1H); ^{13}C NMR (126 MHz, CD_3CN) δ 167.4, 165.9, 147.2, 135.5, 133.4, 131.5, 129.3, 127.1, 122.8, 115.3, 66.8, 54.5, 48.9, 47.4, 39.1, 30.8.

*(E)-2-((R)-1-((S)-1-(2-Chlorophenyl)-2-methoxy-2-oxoethyl)-4-mercaptopiperidin-3-ylidene)acetic Acid Trifluoroacetate (3E,4R,7S)-**3** (H2).* Prepared as for H1 (0.034 g, 72%). IR (CD_3CN, cm^{-1}) 2565, S−H; 1790, C═O; 1753, C═O; 1727, C═O; 1199, C−O; 1168, C−O; HRMS (ESI+) *m*/*z* Calcd for $C_{16}H_{19}ClNO_4S$ [M + H^+] 356.0723, found 356.0716 (ion trap); ^1H NMR (500 MHz, CD_3CN) δ 7.66−7.61 (m, 2H), 7.58 (td, *J* = 7.8, 1.8 Hz, 1H), 7.53 (dd, *J* = 7.7, 1.4 Hz, 1H), 6.34 (d, *J* = 1.1 Hz, 1H), 5.57 (s, 1H), 4.68 (s, 2H), 4.02 (t, *J* = 5.4 Hz, 1H), 3.80 (s, 3H), 3.50 (ddd, *J* = 12.6, 8.9, 3.3 Hz, 1H), 3.38 (ddd, *J* = 12.9, 7.2, 3.9 Hz, 1H), 2.49 (ddt, *J* = 15.2, 9.0, 4.3 Hz, 1H), 2.03−1.99 (m, 1H); ^{13}C NMR (126 MHz, CD_3CN) δ 167.3, 166.0, 147.2, 135.5, 133.4, 131.6. 131.5, 129.3, 127.1, 122.8, 66.7, 54.5, 49.1, 48.7, 39.3, 31.3.

*(Z)-2-((S)-1-((S)-1-(2-Chlorophenyl)-2-methoxy-2-oxoethyl)-4-mercaptopiperidin-3-ylidene)acetic acid formate (3Z,4S,7S)-**3** (H3).* Prepared as for H1, except ammonium acetate was used in place of TFA in the quench and HPLC eluent (0.026 g, 66%, contaminated with trace H4). IR ($CDCl_3$, cm^{-1}) 2572, S−H; 1744, C═O; 1689, C═O; 1648, C═O; 1201, C−O; 1167, C−O; HRMS (ESI+) *m*/*z* Calcd for $C_{16}H_{19}ClNO_4S$ [M + H^+] 356.0723, found 356.0716 (ion trap); ^1H NMR (601 MHz, $CDCl_3$) δ 7.58 (dd, *J* = 7.3, 2.0 Hz, 1H), 7.41 (dd, *J* = 7.3, 1.7 Hz, 1H), 7.33−7.23 (m, 2H), 5.48 (s, 1H), 5.26 (t, *J* = 4.9 Hz, 1H), 4.81 (s, 1H), 3.71 (s, 3H), 3.55 (d, *J* = 13.0 Hz, 1H), 3.01 (d, *J* = 13.0 Hz, 1H), 2.92−2.84 (m, 1H), 2.83−2.75 (m, 1H), 2.28−2.19 (m, 1H), 2.12 (d, *J* = 6.1 Hz, 1H), 1.84 (dq, *J* = 14.4, 1.9 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.9, 170.3, 159.6, 134.7, 132.9, 130.0, 129.8, 129.7, 127.2, 114.0, 67.9, 53.5, 52.3, 45.6, 32.8, 31.9.

*(Z)-2-((R)-1-((S)-1-(2-Chlorophenyl)-2-methoxy-2-oxoethyl)-4-mercaptopiperidin-3-ylidene)acetic Acid Formate (3Z,4R,7S)-**3** (H4).* Prepared as for H1, except ammonium acetate was used in place of TFA in the quench and HPLC eluent (0.024 g, 62%). IR ($CDCl_3$, cm^{-1}) 2567, S−H; 1740, C═O; 1689, C═O; 1648, C═O; 1204, C−O; 1166, C−O; HRMS (ESI+) *m*/*z* Calcd for $C_{16}H_{19}ClNO_4S$ [M + H^+] 356.0723, found 356.0723 (ion trap); ^1H NMR (601 MHz, $CDCl_3$) δ 7.61−7.56 (m, 1H), 7.43−7.39 (m, 1H), 7.32−7.23 (m, 2H), 5.60 (s, 1H), 5.27 (t, *J* = 5.0 Hz, 1H), 4.82 (s, 1H), 3.72 (s, 3H), 3.63 (d, *J* = 12.7 Hz, 1H), 3.21 (d, *J* = 13.0 Hz, 1H), 2.73−2.64 (m, 2H), 2.24−2.15 (m, 1H), 2.13 (d, *J* = 6.1 Hz, 1H), 1.78 (dq, *J* = 14.2,

DOI: 10.1021/acs.joc.5b00632
J. Org. Chem. 2015, 80, 7019−7032

2.0 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.9, 170.5, 159.3, 134.8, 133.1, 129.9, 129.8, 129.6, 127.2, 114.4, 67.9, 53.7, 52.3, 45.3, 32.8, 31.8

Alternatively, a mixture of all four stereoisomers H1−H4 can be obtained via incubation of 2-oxoclopidogrel **2** with rat S9 in phosphate buffer (pH 7.4) in the presence of NADPH at 37 °C, with reaction monitoring by LC/MS, and stopped upon complete conversion of starting material by mixing with acetonitrile. The isomers can then be separated by repeated preparative HPLC as above.

*Representative Procedure for Derivitization of Clopidogrel Metabolites. (Z)-2-((S)-1-((S)-1-(2-Chlorophenyl)-2-methoxy-2-oxoethyl)-4-(2-(3-methoxyphenyl)-2-oxoethylthio)piperidin-3-ylidene)acetic Acid Trifluoroacetate (3Z,4S,7S)-**13** (MP-H3).* To a solution of (*Z*)-2-((*S*)-4-(acetylthio)-1-((*S*)-1-(2-chlorophenyl)-2-methoxy-2-oxoethyl)piperidin-3-ylidene)acetic acid trifluoroacetate (0.021 g, 0.040 mmol) in MeOH (2.0 mL) was added a freshly prepared 1 M solution of NaOH in MeOH (0.12 mL). After 30 min the resultant solution was added to 0.1 M pH 7.4 buffer (18 mL), and 2-bromo-1-(3-methoxyphenyl)ethanone (0.009 g, 0.004 mmol) dissolved in acetonitrile (1.8 mL) was added. After 30 min, the reaction was extracted into EtOAc, and the organic layer was concentrated and purified by HPLC (Luna 5 μm C18 30 × 250 mm, 10−50% ACN in 0.1% TFA/water) to furnish (*Z*)-2-((*S*)-1-((*S*)-1-(2-chlorophenyl)-2-methoxy-2-oxoethyl)-4-(2-(3-methoxyphenyl)-2-oxoethylthio)piperidin-3-ylidene)acetic acid trifluoroacetate (3*Z*,4*S*,7*S*)-**13** (0.018 g, 0.030 mmol, 74% yield). Calcd for $C_{25}H_{27}ClNO_6S$ [M + H$^+$] 504.1248, found 504.1231 (ion trap); IR (neat, cm^{-1}) 1753, C═O; 1706, C═O; 1672, C═O; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.64−7.30 (m, 7H), 7.11 (dd, *J* = 8.3, 2.8 Hz, 1 H), 5.97 (s, 1H), 5.70 (s, 1H), 5.22 (d, *J* = 3.3 Hz, 1H), 4.22−4.07 (m. 3H), 3.87 (br s, 1H), 3.84 (s, 3H), 3.78 (s, 3H), 3.59 (d, *J* = 11.0 Hz, 1H), 3.29−3.18 (m, 1H), 2.86−2.73 (m, 1H), 2.07 (d, *J* = 14.8 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 194.4, 167.5, 166.3, 159.8, 136.9, 135.9, 132.1, 131.4, 131.0, 129.8, 128.0, 122.1, 121.0, 120.1, 112.5, 66.5, 55.4, 53.5, 51.6, 48.2, 39.6, 38.0, 28.6.

*(Z)-2-((R)-4-(Acetylthio)-1-((S)-1-(2-chlorophenyl)-2-methoxy-2-oxoethyl)piperidin-3-ylidene)acetic Acid Trifluoroacetate (3Z,4R,7S)-**13** (MP-H4).* (70%) Calcd for $C_{25}H_{27}ClNO_6S$ [M + H$^+$] 504.1248, found 504.1234 (ion trap); IR (neat, cm^{-1}) 1745, C═O; 1678, C═O; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.67 (dd, *J* = 7.2, 1.7 Hz, 1H), 7.58−7.53 (m, 1H), 7.50−7.40 (m, 4H), 7.36 (t, *J* = 7.7 Hz, 1H), 7.12 (dd, *J* = 8.3, 2.8 Hz, 1H), 6.03 (s, 1H), 5.5 (s, 1H), 5.03 (d, *J* = 2.8 Hz, 1H), 4.32−4.10 (m, 3H), 3.88−3.83 (m, 4H), 3.80 (s, 3H), 3.38 (d, *J* = 12.6 Hz, 1H), 3.18 (t, *J* = 11.3 Hz, 1H), 2.70−2.58 (m, 1H), 2.09−2.02 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 194.6, 167.2, 166.7, 159.9, 145.6, 136.9, 135.6, 132.0, 130.8, 129.8, 128.3, 127.1, 122.39, 121.0, 120.2, 112.5, 66.7, 55.5, 54.5, 53.6, 46.1, 39.5, 38.7, 28.4

*(E)-2-((S)-4-(Acetylthio)-1-((S)-1-(2-chlorophenyl)-2-methoxy-2-oxoethyl)piperidin-3-ylidene)acetic Acid Trifluoroacetate (3E,4S,7S)-**13** (MP-H1).* (61%) Calcd for $C_{25}H_{27}ClNO_6S$ [M + H$^+$] 504.1248, found 504.1237 (ion trap); IR (neat, cm^{-1}) 1750, C═O; 1670, C═O; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.72−7.67 (m, 1H), 7.55−7.34 (m, 6H), 7.14 (dd, *J* = 7.7, 2.2 Hz, 1H), 6.21 (s, 1H), 5.57 (s, 1H), 5.27 (d, *J* = 13.7 Hz, 1H), 4.19 (d, *J* = 13.7 Hz, 1H), 3.94 (d, *J* = 15.4 Hz, 1H), 3.85 (s, 3H), 3.82 (d, *J* = 3.3 Hz, 1H), 3.77 (s, 3H), 3.61−3.45 (m, 2H), 3.29 (d, *J* = 12.1 Hz, 1H), 2.69−2.56 (m, 1H), 2.02 (d, *J* = 13.2 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 193.7, 167.2, 166.7, 160.0, 144.1, 136.4, 135.2, 132.0, 130.9, 130.8, 129.8, 128.4, 127.1, 123.5, 121.2, 120.4, 112.9, 64.9, 55.5, 53.6, 46.7, 45.7, 44.8, 36.3, 27.9.

*(E)-2-((R)-4-(Acetylthio)-1-((S)-1-(2-chlorophenyl)-2-methoxy-2-oxoethyl)piperidin-3-ylidene)acetic Acid Trifluoroacetate (3E,4R,7S)-**13** (MP-H2).* (73%) Calcd for $C_{25}H_{27}ClNO_6S$ [M + H$^+$] 504.1248, found 504.1232 (ion trap); IR (neat, cm^{-1}) 1754, C═O; 1673, C═O; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.69 (dd, *J* = 6.9, 1.9 Hz, 1H), 7.56−7.33 (m, 6H), 7.15 (dd, *J* = 8.0, 1.9 Hz, 1H), 6.14 (s, 1H), 5.46 (s, 1H), 5.11 (d, *J* = 13.2 Hz, 1H), 4.04 (d, *J* = 13.2 Hz, 1H), 3.93 (d, *J* = 15.4 Hz, 1H), 3.86 (s, 3H), 3.84−3.79 (m, 3H), 3.84−3.79 (m, 4H), 3.77 (br s, 1H), 3.62 (d, *J* = 15.4 Hz, 1H), 3.33 (d, *J* = 11.5 Hz, 1H), 3.22−3.11 (m, 1H), 2.73−2.60 (m, 1H), 2.03 (dd, *J* = 14.8, 2.8 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 193.7, 167.2, 160.0, 136.5, 135.4, 131.9, 130.9, 130.7, 129.8, 128.3, 127.4, 122.8, 121.1, 120.4, 112.8, 66.8, 55.5, 53.7, 47.9, 46.4, 45.0, 36.6, 28.6

*6-Methoxy-2,2-dimethyldihydrofuro[3,4-d][1,3]dioxol-4(3aH)-one (3aS,6R,6aR)-(−)-**29**.* A solution of D-(−)-ribose (20.0 g, 133. mmol) and 2,2-dimethoxypropane (40.0 mL, 326 mmol) in acetone (160 mL) was cooled to 0 °C. Perchloric acid (aq, 70%, 8 mL, 90 mmol) was added dropwise. The ice bath was removed, and the solution was stirred at room temperature for 2 h. MeOH (28 mL, 690 mmol) was then added, and the solution was stirred for an additional 2 h. The bright yellow solution was cooled and aqueous sodium bicarbonate (12.8 g in 40 mL H_2O) was added carefully, causing a white solid to precipitate, which was filtered off. The filtrate was evaporated to a final volume of 60 mL and extracted with Et_2O (2 × 300 mL). The combined organic fractions were washed with brine (100 mL) and dried over magnesium sulfate. The solvents were evaporated, and protected D-(−)-ribose (24.8 g, 91%) was obtained as a yellow oil. Without further purification, the oil (4.00 g, 19.6 mmol) was dissolved in benzene (200 mL) in a 500 mL flask attached to a water separator and a condenser. Pyridinium chlorochromate (16.8 g, 77.9 mmol) was added under stirring. The reaction mixture was refluxed for 12 h. The solvent was decanted from the black solid, and the residue was extracted with Et_2O (3 × 50 mL). The combined organic fractions were filtered with a Büchner funnel, filtered through a Celite/silica plug, concentrated to a final volume of 50 mL. After evaporation of the solvents in vacuo, a white solid in a green semisolid remained, which was filtered again through paper and through a plug of Celite/silica. After evaporation of the solvents, a clear oil was obtained. The residue was purified by flash chromatography (hexanes/EtOAc, 9:1 to 4:1). (−)-**29** (1.2 g, 30%) was obtained as white needles. The spectroscopic data for this compound were identical to those reported in ref 18a.

*6-Methoxy-2,2-dimethyldihydrofuro[3,4-d][1,3]dioxol-4(3aH)-one (3aR,6S,6aS)-(+)-**29**.* (+)-**29** was prepared with the same procedure as for (−)-**29**, starting with L-(+)-ribose instead of D-(−)-ribose.

*2,2-Dimethyl-3a,6a-dihydro-4H-cyclopenta[d][1,3]dioxol-4-one (3aS,6aS)-(+)-**30**.* Dimethyl methylphosphonate (1.0 mL, 9.4 mmol) was dissolved in THF (60 mL) under Ar, and the solution was cooled to −78 °C. *n*-BuLi (2.5 M in hexane, 3.8 mL, 9.5 mmol) was added dropwise over 10 min, and the solution was stirred for 15 min. A solution of (−)-**29** (1.8 g, 9.4 mmol) in THF (10 mL) was added rapidly, and the solution was stirred for 2.5 h at −78 °C. After the mixture was slowly warmed to room temperature (45 min) and stirred at room temperature for 30 min, the solution became yellow. Then a mixture of Et_2O/H_2O (5:1, 150 mL) was added. The phases were separated, and the aqueous phase was extracted with ether (2 × 50 mL). The combined organic fractions were washed with brine and dried over sodium sulfate. The solvent was concentrated, and a yellow oil was obtained. The oil was purified by flash chromatography (hexanes/EtOAc, 19:1 to 9:1) to afford (+)-**30** (620 mg, 43%) as white crystals. The spectroscopic data for this compound were identical to those reported in ref 18a.

*2,2-Dimethyl-3a,6a-dihydro-4H-cyclopenta[d][1,3]dioxol-4-one (3aR,6aR)-(−)-**30**.* (−)-**30** was prepared with the same procedure as for (+)-**30**, starting with (+)-**29** instead of (−)-**29**.

*2,2-Dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-one (3aS,6aS)-(+)-**27**.* (+)-**30** (420 mg, 2.72 mmol) was dissolved in ethyl acetate (7 mL), and palladium on activated carbon (42 mg, 10% weight) was added under stirring. The solution was flushed with N_2 and then with H_2. The reaction was stirred under a hydrogen atmosphere (maintained with a balloon) overnight. The mixture was filtered through a Celite plug and concentrated in vacuo to afford pure (+)-**27** (415 mg, 98%) as white crystals. The spectroscopic data for this compound were identical to those reported in ref 18b.

*2,2-Dimethyl-3a,6a-dihydro-4H-cyclopenta[d][1,3]dioxol-4-one (3aR,6aR)-(−)-**30**.* (−)-**30** was prepared with the same procedure as for (+)-**30**, starting with (+)-**29** instead of (−)-**29**.

*tert-Butyl 2-(2,2-Dimethyltetrahydro-4H-cyclopenta[d][1,3]-dioxol-4-ylidene)acetate (3aR,6aS)-**32**.* To a mechanically stirred

DOI: 10.1021/acs.joc.5b00632
J. Org. Chem. 2015, 80, 7019−7032

suspension of NaH (92 mg, 3.83 mmol, washed with hexane) in THF (20 mL) was added at 0 °C under Ar *tert*-butyl (diethoxyphosphoryl)-acetate (856 mg, 3.87 mmol) over 40 min. To the colorless solution was added a solution of (+)-**27** (597 mg, 382 mmol) in THF (5 mL). After 1 h at room temperature, the solvent was evaporated, the residual gel was diluted with potassium phosphate monobasic aqueous solution, and the mixture was extracted with Et_2O. The combined organic phase was dried over magnesium sulfate and evaporated, and the residual oil was purified by flash chromatography (hexanes/EtOAc, 19:1). **32** (809 mg, 84%, 1:1 inseparable diastereoisomeric mixture) was obtained as a colorless oil. The mixture was used for the next step. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.90−5.87 (m, 1H), 5.82−5.78 (m, 1H), 5.48 (d, J = 5.6 Hz, 1H), 4.77−4.71 (m, 2H), 4.68 (t, J = 4.8 Hz, 1H), 3.09 (dd, J = 17.9, 8.1 Hz, 1H), 2.82−2.68 (m, 2H), 2.22 (dd, J = 15.6, 7.1 Hz, 1H), 2.08 (dd, J = 14.1, 8.4 Hz, 1H), 1.98 (dd, J = 13.8, 7.8 Hz, 1H), 1.75−1.62 (m, 1H), 1.60−1.45 (m, 1H), 1.49 (s, 9H), 1.47 (s, 9H), 1.45 (s, 3H), 1.43 (s, 3H), 1.37 (s, 3H), 1.34 (s, 3H).

Enantiomers of *tert*-butyl 2-(2,2-dimethyltetrahydro-4*H*-cyclopenta-[*d*][1,3]dioxol-4-ylidene)acetate (3a*R*,6a*S*)-**32** were prepared with the same procedure as for **32**, starting with (−)-**27** instead of (+)-**27**.

*tert-Butyl (E)-2-(5-Hydroxy-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-ylidene)acetate (3aR,5S,6aS)-(+)-**34** and (Z)-2-(5-Hydroxy-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-ylidene)acetate (3aR,5S,6aS)-(+)-**35**.* Selenium dioxide (198 mg, 1.79 mmol) was added under stirring to a solution of **32** (351 mg, 1.38 mmol) in dioxane (20 mL). The mixture was heated at 100 °C and stirred for 3 h. The reaction mixture was cooled to room temperature, and then the suspension was diluted with EtOAc and successively washed with 5% sodium bicarbonate aqueous solution, 5% aqueous copper sulfate, 10% aqueous sodium thiosulfate, and brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to afford a red oil. The oil was purified by flash chromatography (hexanes/EtOAc, 19:1 to 9:1). (+)-**34** was obtained as an orange oil (111 mg, 30%), and (+)-**35** was obtained as orange crystals (87 mg, 23%).

(+)-**34**: $[\alpha]_D$ +201° (c 2.0, CH_2Cl_2); R_f = 0.5 (silica gel, EtOAc/hexanes 3:7); HRMS (ESI$^+$) m/z Calcd for $C_{14}H_{22}O_5$ [M + Na$^+$] 293.1359, found 293.1364; IR (cm^{-1}) 3442, 2982, 2935, 2361, 2340, 1687, 1369, 1242, 1150, 1077, 855; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.06−6.02 (m, 1 H), 5.39 (d, J = 1.5 Hz, 1H), 5.29−5.19 (m, 1H), 4.85 (dd, J = 3.7, 1.7 Hz, 1H), 4.69 (t, J = 5.1, 5.1 Hz, 1H), 2.55 (dd, J = 14.6, 8.1 Hz, 1H), 1.86 (ddd, J = 14.3, 9.1, 4.9 Hz, 1H), 1.49 (s, 9H), 1.39 (s, 3H), 1.32 (s, 3H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 167.5, 165.9, 122.1, 111.7, 82.9, 81.8, 78.1, 70.8, 36.9, 28.2, 27.4, 25.4.

(+)-**35**: Mp 90−92 °C; $[\alpha]_D$ +203° (c 2.0, CH_2Cl_2); R_f = 0.3 (silica gel, EtOAc/hexanes 3:7); HRMS (ESI$^+$) m/z Calcd for $C_{14}H_{22}O_5$ [M + Na$^+$] 293.1359, found 293.1367; IR (cm^{-1}) 3476, 2980, 2935, 1712, 1369, 1237, 1210, 1169, 1138, 1046, 858; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.99 (dd, J = 2.3, 1.3 Hz, 1H), 5.57 (d, J = 6.1 Hz, 1H), 4.91 (dtd, J = 8.9, 7.3, 7.2, 1.6 Hz, 1H), 4.67 (t, J = 5.7, 5.7 Hz, 1H), 2.41 (dd, J = 13.5, 7.4 Hz, 1H), 1.50 (s, 9H), 1.48−1.44 (m, 1H), 1.44 (s, 3H), 1.36 (s, 3H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 164.9, 159.7, 118.5, 110.6, 80.9, 76.6, 71.9, 37.9, 28.3, 26.5, 24.2.

*tert-Butyl (E)-2-(5-Hydroxy-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-ylidene)acetate (3aS,5R,6aR)-(−)-**34** and (Z)-2-(5-Hydroxy-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-ylidene)acetate (3aS,5R,6aR)-(−)-**35**.* (−)-**34** and (−)-**35** were prepared with the same procedure as for (+)-**34** and (−)-**35**, starting with the enantiomeric mixture of **32** instead of **32**. (−)-**34**: $[\alpha]_D$ −201°. (−)-**36**: $[\alpha]_D$ −203°.

*Methyl (S)-2-((S,Z)-3-(2-(tert-Butoxy)-2-oxoethylidene)-4-hydroxypiperidin-1-yl)-2-(2-chlorophenyl)acetate (+)-**38**.* (+)-**34** (89 mg, 0.33 mmol) was dissolved in 10 mL of dichloromethane, and Montmorillonite K10-Clay (890 mg) was added under vigorous stirring. The reaction was stirred at room temperature during 1 h. The suspension was diluted in methanol and filtered through a silica plug by adding methanol to wash the Montmorillonite K10-Clay. The solvents were concentrated in vacuo to afford the corresponding triol as a red/orange oil. To a vigorously stirred solution of the crude triol in dichloromethane (10 mL) cooled to 0 °C was added the silica gel-supported periodate reagent (912 mg, prepared as described in ref 18). After 5 min, the mixture was diluted with EtOAc and filtered through a silica plug, and the silica gel was thoroughly washed with EtOAc. The solvents were concentrated in vacuo to afford dialdehyde **36** as a yellow oil. The crude dialdehyde was subsequently dissolved in a mixture of ACN/MeOH (19:1, 30 mL). L-(+)-2-Chlorophenylglycine **17** (78 mg, 0.33 mmol) and sodium cyanoborohydride (42 mg, 0.66 mmol) were added under stirring. The reaction was stirred for 2 h, and solvents were concentrated in vacuo. Then a mixture of EtOAc and aqueous saturated sodium bicarbonate (1:1, 60 mL) was added. The phases were separated, and the aqueous phase was extracted with ether (5 × 20 mL). Combined organic fractions were dried over magnesium sulfate. The solvent was concentrated in vacuo, and a yellow oil was obtained. The oil was purified by flash chromatography (gradient hexane/EtOAc, 19:1 to 9:1). Compound (+)-**38** (40 mg, 31%) was obtained as a pale oil. $[\alpha]^{20.0}_D$ +40° (c 2.7, CH_2Cl_2); R_f = 0.4 (silica gel, EtOAc/hexanes 3:7); HRMS (ESI$^+$) m/z Calcd for $C_{20}H_{26}ClNO_5$ [M + H$^+$] 396.1572, found 396.1572; IR (cm^{-1}) 3405, 2926, 1743, 1711, 1367, 1256, 1145, 1039, 1020, 754; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.63−7.58 (m, 1H), 7.40−7.38 (m, 1H), 7.31−7.22 (m, 2H), 5.58 (s, 1H), 5.01−4.94 (m, 1H), 4.76 (s, 1H), 4.04 (br s, 1H), 3.70 (s, 3H), 3.29 (d, J = 12.8 Hz, 1 H), 2.96 (d, J = 12.8 Hz, 1H), 2.82−2.76 (m, 1H), 2. 69−2.59 (m, 1H), 2.03−1.96 (m, 1H),1.92−1.85 (m, 1H), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 171.3, 166.9, 155.4, 134.8, 133.4, 130.0, 130.0, 129.6, 127.3, 118.8, 81.6, 67.9, 66.2, 56.0, 52.3, 47.3, 33.0, 28.2.

Methyl (S)-2-((S,E)-3-(2-(tert-Butoxy)-2-oxoethylidene)-4-hydroxypiperidin-1-yl)-2-(2-chlorophenyl)acetate. (+)-**39** was prepared with the same three-step procedure as for (+)-**38**, starting with (+)-**35** (57 mg, 0.21 mmol) instead of (+)-**34**. Flash chromatography (gradient hexane/ethyl acetate, 19:1 to 9:1) afforded (+)-**39** (48 mg, 58%) as a pale oil. $[\alpha]_D$ +7° (c 2.2, CH_2Cl_2); R_f = 0.2 (silica gel, EtOAc/hexanes 3:7); HRMS (ESI$^+$) m/z Calcd for $C_{20}H_{26}ClNO_5$ [M + H$^+$] 396.1572, found 396.1576; IR (cm^{-1}) 3430, 2927, 1740, 1709, 1367, 1217, 1164, 1146, 754; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.59 (d, J = 7.4 Hz, 1H), 7.37 (d, J = 7.6 Hz, 1H), 7.31−7.22 (m, 2H), 5.88 (s, 1H), 4.81 (s, 1H), 4.49 (d, J = 13.9 Hz, 1H), 4.13 (br s, 1H), 3.72 (s, 3H), 3.22 (d, J = 13.9 Hz, 1H), 2.96−2.87 (m, 1H), 2.59−2.45 (m, 1H), 2.07−1.99 (m, 1H),1.85−1.76 (m, 1H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 171.2, 165.6, 154.4, 134.8, 133.7, 130.1, 129.9, 129.5, 127.2, 115.8, 80.5, 71.4, 67.8, 52.3, 49.8, 47.8, 34.2, 28.3.

Methyl (S)-2-((R,Z)-3-(2-(tert-Butoxy)-2-oxoethylidene)-4-hydroxypiperidin-1-yl)-2-(2-chlorophenyl)acetate. (−)-**47** was prepared with the same three-step procedure as for (+)-**38**, starting with (−)-**34** (63 mg, 0.23 mmol) instead of (+)-**34**. Flash chromatography (gradient hexane/ethyl acetate, 19:1 to 9:1) afforded (−)-**47** (17 mg, 18%) as a pale oil. $[\alpha]_D$ −7.8° (c 2.7, CH_2Cl_2); R_f = 0.4 (silica gel, EtOAc/hexanes 3:7); HRMS (ESI$^+$) m/z Calcd for $C_{20}H_{26}ClNO_5$ [M + H$^+$] 396.1572, found 396.1577; IR (cm^{-1}) 3419, 2977, 1743, 1711, 1368, 1250, 1145, 755; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.61 (dd, J = 7.5, 1.9 Hz, 1H), 7.39 (dd, J = 7.7, 1.5 Hz, 1H), 7.31−7.22 (m, 2H), 5.63 (s, 1H), 5.01 (t, J = 4.8 Hz, 1H), 4.76 (s, 1H), 3.91 (br s, 1H), 3.70 (s, 3H), 3.29 (d, J = 12.6 Hz, 1H), 3.08 (d, J = 13.9 Hz, 1H), 2.83−2.76 (m, 1H), 2.56−2.49 (m, 1H), 2.00−1.92 (m, 1H), 1.89−1.83 (m, 1H), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 171.2, 166.8, 155.3, 134.8, 133.5, 130.0, 130.0, 129.6, 127.3, 118.9, 81.5, 68.0, 65.9, 56.4, 52.4, 46.7, 33.0, 28.2.

Methyl (S)-2-((R,E)-3-(2-(tert-Butoxy)-2-oxoethylidene)-4-hydroxypiperidin-1-yl)-2-(2-chlorophenyl)acetate. (+)-**48** was prepared with the same three-step procedure as for (+)-**38**, starting with (−)-**35** (64 mg, 0.24 mmol) instead of (+)-**35**. Flash chromatography (gradient hexane/EtOAc, 19:1 to 9:1) afforded (+)-**48** (34 mg, 38%) as a pale oil. $[\alpha]_D$ +62° (c 2.7, CH_2Cl_2); R_f = 0.2 (silica gel, EtOAc/hexanes 3:7); HRMS (ESI$^+$) m/z Calcd for $C_{20}H_{26}ClNO_5$ [M + H$^+$] 396.1572, found 396.1571; IR (cm^{-1}) 3407, 2952, 1740, 1708, 1366, 1219, 1144, 753, 729; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.58−7.53 (m, 1H), 7.41−7.37 (m, 1H), 7.31−7.22 (m, 2H), 5.92 (s, 1H), 4.86 (s, 1H), 4.45 (d, J = 13.9 Hz, 1H), 4.14 (dd, J = 8.0, 5.3 Hz, 1H), 3.71 (s, 3H), 3.26 (d, J = 13.9 Hz, 1H), 2.90 (dtd, J = 11.7, 4.7, 2.0 Hz, 1H), 2.65 (ddd, J = 12.0, 9.9, 3.9 Hz, 1H), 2.23 (br s, 1H), 2.01 (ddd, J =

DOI: 10.1021/acs.joc.5b00632
J. Org. Chem. 2015, 80, 7019−7032

13.5, 9.1, 4.6 Hz, 1H), 1.84−1.74 (m, 1H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 171.3, 165.6, 154.6, 134.7, 133.7, 130.1, 130.0, 129.5, 127.2, 115.7, 80.4, 71.4, 67.1, 52.3, 50.1, 47.0, 34.3, 28.2.

Methyl (S)-2-((R,Z)-4-(Acetylthio)-3-(2-(tert-butoxy)-2-oxoethylidene)piperidin-1-yl)-2-(2-chlorophenyl)acetate (+)-21. Compound (+)-**38** (12 mg, 0.030 mmol) was dissolved in dichloromethane (2.5 mL) under argon, and the solution was cooled to 0 °C. Triethylamine (48 μL, 0.35 mmol) and methanesulfonyl chloride (24 μL, 0.31 mmol) were added under stirring. After the mixture was stirrred 1 h at 0 °C, it was diluted with EtOAc (50 mL) and filtered through a silica plug, and the silica gel was thoroughly washed with EtOAc. The solvents were concentrated in vacuo to afford the corresponding mesylate as a pale oil. The mesylate was subsequently dissolved in benzene (1 mL). Hünig's base (63 μL, 0.36 mmol) and thioacetic acid (21 μL, 0.30 mmol) were added, and the mixture was left at room temperature for 20 min. The solvent was then concentrated in vacuo, and the mixture was left at room temperature for 5 h. The bright red mixture was filtered through a silica plug, and the silica gel was thoroughly washed with EtOAc. The solvents were concentrated in vacuo to afford a yellow oil. The oil was purified by flash chromatography (gradient hexane/EtOAc, 19:1 to 9:1) to afford the corresponding thioacetate. This thioacetate was dissolved in a mixture trifluoroacetic acid/dichloromethane (0.5 mL/1.0 mL), and the reaction was left at room temperature for 1 h. The solvents were concentrated, and the resulting oil was purified by HPLC (eluting at 42.5 mL/min with a gradient starting at 15% ACN/H_2O (0.1% TFA) and concluding at 95% ACN/H_2O over 40 min) to afford (+)-**21** as a pale oil after basifying with sodium bicarbonate (4 mg, 38% over three steps). $[\alpha]_D$ +35.6° (*c* 0.5, CH_2Cl_2). All spectroscopical data were identical to those obtained with the stereodivergent route.

Methyl (S)-2-((R,E)-4-(Acetylthio)-3-(2-(tert-butoxy)-2-oxoethylidene)piperidin-1-yl)-2-(2-chlorophenyl)acetate. (+)-**23** was prepared with the same three-step procedure as for (+)-**21**, starting with (+)-**39** (9.0 mg, 0.023 mmol) instead of (+)-**38**. HPLC (eluting at 42.5 mL/min with a gradient starting at 15% ACN/H_2O (0.1% TFA) and concluding at 95% ACN/H_2O over 40 min) afforded (+)-**23** as a pale oil after basifying with sodium bicarbonate (6 mg, 65% over three steps). $[\alpha]_D$ +83.2° (*c* 0.5, CH_2Cl_2). All spectroscopical data were identical to the ones obtained with the stereodivergent route.

Methyl (S)-2-((S,Z)-4-(Acetylthio)-3-(2-(tert-butoxy)-2-oxoethylidene)piperidin-1-yl)-2-(2-chlorophenyl)acetate. (+)-**20** was prepared with the same three-step procedure as for (+)-**21**, starting with (−)-**47** (14 mg, 0.035 mmol) instead of (+)-**38**. HPLC (eluting at 42.5 mL/min with a gradient starting at 15% ACN/H_2O (0.1% TFA) and concluding at 95% ACN/H_2O over 40 min) afforded (+)-**21** as a pale oil after basifying with sodium bicarbonate (4 mg, 29% over three steps). $[\alpha]_D$ +57.6° (*c* 0.5, CH_2Cl_2). All spectroscopical data were identical to those obtained with the stereodivergent route.

Methyl (S)-2-((S,E)-4-(Acetylthio)-3-(2-(tert-butoxy)-2-oxoethylidene)piperidin-1-yl)-2-(2-chlorophenyl)acetate. (+)-**22** was prepared with the same three-step procedure as for (+)−**21**, starting with (+)-**48** (22 mg, 0.055 mmol) instead of (+)-**38**. HPLC (eluting at 42.5 mL/min with a gradient starting at 15% ACN/H_2O (0.1% TFA) and concluding at 95% ACN/H_2O over 40 min) afforded (+)-**22** as a pale oil after basifying with sodium bicarbonate (15 mg, 69% over three steps). $[\alpha]_D$ +34.0° (*c* 0.5, CH_2Cl_2). All spectroscopical data were identical to those obtained with the stereodivergent route.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra and selected cif data for all new compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b00632.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: scott.shaw@bms.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Dr. M. Dabros (BMS), Prof. A. Rheingold, and Dr. C. E. Moore (UCSD) for X-ray crystallographic analysis, Dr. D.-H. Huang and Dr. L. Pasternack (TSRI) for assistance with NMR spectroscopy, Dr. A. Apedo and Dr. O. McConnell (BMS) for assistance with IR spectroscopy, Dr. R. Langish (BMS) for assistance with mass spectrometry, and Dr. E. Kick (BMS) for valuable discussion regarding the preparation of this manuscript.

■ REFERENCES

(1) (a) Pereillo, J. M.; Maftouh, M.; Andrieu, A.; Uzabiaga, M. F.; Fedeli, O.; Savi, P.; Pascal, M.; Herbert, J. M.; Maffrand, J. P.; Picard, C. *Drug Metab. Dispos.* **2002**, *30*, 1288. Therein metabolites **3** were designated trivial names H1−H4 on the basis of HPLC retention times, and these names are used throughout this text. (b) Yoneda, K.; Iwamura, R.; Kishi, H.; Mizukami, Y.; Mogami, K.; Kobayashi, S. *Br. J. Pharmacol.* **2004**, *142*, 551. (c) Hasegawa, M.; Sugidachi, A.; Ogawa, T.; Isobe, T.; Jakubowski, J. A.; Asai, F. *Thromb. Haemost.* **2005**, *94*, 593. (d) Farid, N. A.; Kurihara, A.; Wrighton, S. A. *J. Clin. Pharmacol.* **2010**, *50*, 126.

(2) Brandt, J. T.; Close, S. L.; Iturria, S. J.; Payne, C. D.; Farid, N. A.; Ernest, C. S., II; Lachno, D. R.; Salazar, D.; Winters, K. J. *J. Thromb. Haemost.* **2007**, *5*, 2429.

(3) Kazui, M.; Nishiya, Y.; Ishizuka; Hagihara, K.; Farid, N. A.; Okazaki, O.; Ikeda, T.; Kurihara, A. *Drug Metab. Dispos.* **2010**, *38*, 92.

(4) Dansette, P. M.; Libraire, J.; Bertho, G.; Mansuy, D. *Chem. Res. Toxicol.* **2009**, *22*, 369.

(5) Hagihara, K.; Kazui, M.; Kurihara, A.; Yoshiike, M.; Honda, K.; Okazaki, O.; Farid, N. A.; Ikeda, T. *Drug. Metab. Dispos.* **2009**, *37*, 2145.

(6) Shuldiner, A. R.; O'Connell, J. R.; Bliden, K. P.; Gandhi, A.; Ryan, K.; Horenstein, R. B.; Damcott, C. M.; Pakyz, R.; Tantry, U. S.; Gibson, Q.; Pollin, T. I.; Post, W.; Parsa, A.; Mitchell, B. D.; Faraday, N.; Herzog, W.; Gurbel, P. A. *J. Am. Med. Assoc.* **2009**, *302*, 849.

(7) (a) Hulot, J. S.; Bura, A.; Villard, E.; Azizi, M.; Remones, V.; Goyenvalle, C.; Aiach, M.; Lechat, P.; Gaussem, P. *Blood* **2006**, *108*, 2244. (b) Collet, J. P.; Hulot, J. S.; Pena, A.; Villard, E.; Esteve, J. B.; Silvain, J.; Payot, L.; Brugier, D.; Cayla, G.; Beygui, F.; Bensimon, G.; Funck-Brentano, C.; Montalescot, G. *Lancet* **2009**, *373*, 309. (c) Mega, J. L.; Close, S. L.; Wiviott, S. D.; Shen, L.; Hockett, R. D.; Brandt, J. T.; Walker, J. R.; Antman, E. M.; Macias, W.; Braunwald, E.; Sabatine, M. S. *N. Engl. J. Med.* **2009**, *360*, 354.

(8) (a) Gong, I. Y.; Crown, N.; Suen, C. M.; Schwarz, U. I.; Dresser, G. K.; Knauer, M. J.; Sugiyama, D.; DeGorter, M. K.; Woolsey, S.; Tirona, R. G.; Kim, R. B. *Eur. Heart. J.* **2012**, *33*, 2856. (b) Simon, T.; Bhatt, D. L.; Bergougnan, L.; Farenc, C.; Pearson, K.; Perrin, L.; Vicaut, E.; Lacreta, F.; Hurbin, F.; Dubar, M. *Clin. Pharmacol. Ther.* **2011**, *90*, 287.

(9) Bouman, H. J.; Schomig; van Werkum, J. W.; Velder, J.; Hackeng, C. M.; Hirschhauser, C.; Waldmann, C.; Schmalz, H. G.; ten Berg, J. M.; Taubert, D. *Nat. Med.* **2011**, *17*, 110.

(10) (a) Hulot, J. S.; Collet, J. P.; Cayla, G.; Silvain, J.; Allanic, F.; Bellemain-Appaix, A.; Scott, S. A.; Montalescot, G. *Circ. Cardiovasc. Interventions* **2011**, *4*, 422. (b) Lewis, J. P.; Fisch, A. S.; Ryan, K.; O'Connell, J. R.; Gibson, Q.; Mitchell, B. D.; Shen, H.; Tanner, K.; Horenstein, R. B.; Pakzy, R.; Tantry, U. S.; Bliden, K. P.; Gurbel, P. A.; Shuldiner, A. R. *Clin. Pharmacol. Ther.* **2011**, *90*, 568. (c) Lewis, J. P.; Shuldiner, A. R. *Circ. Cardiovasc. Genet.* **2012**, *5*, 153.

(11) (a) Dansette, P. M.; Rosi, J.; Bertho, G.; Mansuy, D. *Chem. Res. Toxicol.* **2012**, *25*, 348. (b) Kim, M. J.; Jeong, E. S.; Park, J. S.; Lee, S.

DOI: 10.1021/acs.joc.5b00632
J. Org. Chem. 2015, 80, 7019−7032

J.; Ghim, J. L.; Choi, C. S.; Shin, J. G.; Kim, D. H. *Drug. Metab. Dispos.* **2014**, *42*, 141.
(12) Asai, F.; Sugidachi, A.; Ikeda, T.; Koike, H.; Inoue, T.; Takata, K.; Iwamura, R.; Kita, J.-i.; Yoneda, K. Cyclic Amine Derivatives. U.S. Patent 6,087,379, July 11, 2000.
(13) Subsequent to the completion of this work, a route to the synthesis of MP-H4 was disclosed: Bluet, G.; Blankenstein, J.; Brohan, E.; Prévost, C.; Chevé, M.; Schofield, J.; Roy, S. *Tetrahedron* **2014**, *70*, 3893.
(14) It was anticipated that 10−25 mg of each metabolite stereoisomer and 25−100 mg of each corresponding analyte would be required.
(15) Garber, S. B.; Kingsbury, J. S.; Gray, B. L.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2000**, *122*, 8168.
(16) Parikh, J. R.; Doering, W. v. E. *J. Am. Chem. Soc.* **1967**, *89*, 5505.
(17) Maryanoff, B. E.; Reitz, A. B. *Chem. Rev.* **1989**, *89*, 863.
(18) (a) Klepper, F.; Jahn, E. M.; Hickmann, V.; Carell, T. *Angew. Chem., Int. Ed.* **2007**, *46*, 2325. (b) Smith, A. B., III; Sperry, J. B.; Han, Q. *J. Org. Chem.* **2007**, *72*, 6891.
(19) Zhong, Y.-L.; Shing, T. K. M. *J. Org. Chem.* **1997**, *62*, 2622.
(20) Carpino, L. A. *J. Am. Chem. Soc.* **1960**, *82*, 2725.
(21) *MacroModel*, version 9.5; Schrödinger, L.L.C.: New York, 2007.
(22) *Maestro*, version 8.0; Schrödinger, L.L.C.: New York, 2007.
(23) (a) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648. (b) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
(24) (a) Hehre, W. J.; Ditchfield, R.; Pople, J. A. *J. Chem. Phys.* **1972**, *56*, 2257. (b) Krishnan, R.; Binkley, J. S.; Seeger, R.; Pople, J. A. *J. Chem. Phys.* **1980**, *72*, 650.
(25) Kohn, W.; Sham, L. *J. Phys. Rev.* **1965**, *140*, A1133.
(26) (a) Feyereisen, M.; Fitzgerald, G.; Komornicki, A. *Chem. Phys. Lett.* **1993**, *208*, 359. (b) Weigend, F.; Haser, M.; Patzelt, H.; Ahlrichs, R. *Chem. Phys. Lett.* **1998**, *294*, 143.
(27) Shao, Y.; Fusti-Molnar, L.; Jung, Y.; Kussmann, J.; Ochsenfeld, C.; Brown, S. T.; Gilbert, A. T. B.; Slipchenko, L. V.; Levchenko, S. V.; O'Neill, D. P.; DiStasio, R. A., Jr.; Lochan, R. C.; Wang, T.; Beran, G. J. O.; Besley, N. A.; Herbert, J. M.; Lin, C. Y.; Van Voorhis, T.; Chien, S. H.; Sodt, A.; Steele, R. P.; Rassolov, V. A.; Maslen, P. E.; Korambath, P. P.; Adamson, R. D.; Austin, B.; Baker, J.; Byrd, E. F. C.; Daschel, H.; Doerksen, R. J.; Dreuw, A.; Dunietz, B. D.; Dutoi, A. D.; Furlani, T. R.; Gwaltney, S. R.; Heyden, A.; Hirata, S.; Hsu, C.-P.; Kedziora, G.; Khaliullin, R. Z.; Klunzinger, P.; Lee, A. M.; Lee, M. S.; Liang, W.; Lotan, I.; Nair, N.; Peters, B.; Proynov, E. I.; Pieniazek, P. A.; Rhee, Y. M.; Ritchie, J.; Rosta, E.; Sherrill, C. D.; Simmonett, A. C.; Subotnik, J. E.; Lee Woodcock, H., III; Zhang, W.; Bell, A. T.; Chakraborty, A. K.; Chipman, D. M.; Keil, F. J.; Warshel, A.; Hehre, W. J.; Schaefer, H. F., III; Kong, J.; Krylov, A. I.; Gill, P. M. W.; Head-Gordon, M. *Phys. Chem. Chem. Phys.* **2006**, *8*, 3172.

DOI: 10.1021/acs.joc.5b00632
J. Org. Chem. 2015, 80, 7019−7032