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Synthesis of the stabilized active metabolite of clopidogrel

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ABSTRACT

The convergent synthesis of the stabilized active metabolite of clopidogrel was achieved in eleven steps from commercially available 1,2,3,6-tetrahydropyridine and 2-bromo-3′-methoxy acetophenone (MPBr). This synthetic route used a standard Horner–Wadsworth–Emmons reaction allowing the introduction of a *Z* exocyclic double bond. A selective hydrolysis of an acrylic methyl ester moiety, isolated by an efficient and reliable preparative chiral chromatography at gram scale, released the title compound with a 98% LC purity and d.e. >99%.

1. Introduction

Clopidogrel **1** is an oral antiplatelet agent from the thienopyridine family indicated for the prevention of artherothrombotic events (Fig. 1).[1] Clopidogrel is a P2Y12-ADP receptor antagonist and a prodrug, requiring two distinct metabolic transformations to produce the active metabolite responsible for the anti-aggregating effect.

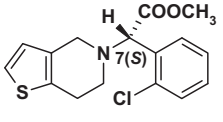

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Fig. 1. Chemical structure of clopidogrel.

The main metabolic pathway converts **1** into an inactive carboxylic acid derivative **2** by hydrolysis (Scheme 1). In vitro studies showed that the pharmacologically active metabolite is generated by a two-step hepatic pathway involving multiple cytochrome P450 isoenzymes.[2] The first step involves oxidation of **1** to the inactive 2-oxo-clopidogrel intermediate **3**. Next, the thiolactone ring is opened, via the sulfinate intermediate, **3′** to give the active metabolite species **4**. Two additional elements of stereogenicity are thus added to the stereocenter already present at position 7: a new stereocenter at position 4 and a stereogenic exocyclic double bond, suggesting that the active metabolite may be one component of a mixture of diastereoisomers.

In vitro metabolism studies on **3** showed that the metabolic transformation led to a mixture of four stereoisomers, referred to as H1, H2, H3 and H4. All four were isolated and characterized: isomers H1 and H2 **4a** are the *E* compounds while isomers H3 and H4 **4b** have *Z* configuration at the exocyclic double bond (Scheme 2).[3] The thiol moiety of the active metabolite **4** binds specifically and irreversibly to cysteine residues of the platelet P2Y12 purinergic receptor, thus inhibiting ADP-mediated platelet activation and aggregation.

In addition, it has been demonstrated by analysis of clinical samples that only metabolites H4 and H3 are formed in vivo, and that H4 was found to be the only biologically active isomer.[4] These various metabolism studies showed that the expression of the anti-aggregant activity of clopidogrel depends on the configuration of the three stereochemical sites: C7, C3–C16 and C4. The *S* configuration at C7 and the *Z* configuration of the C3–C16 double bond are considered to be crucial for expression of activity and only isomers H3 and H4 had these features.

Since only metabolite H4 was active in vivo, it appeared that the activity was also closely linked to the *R* or *S* configuration at

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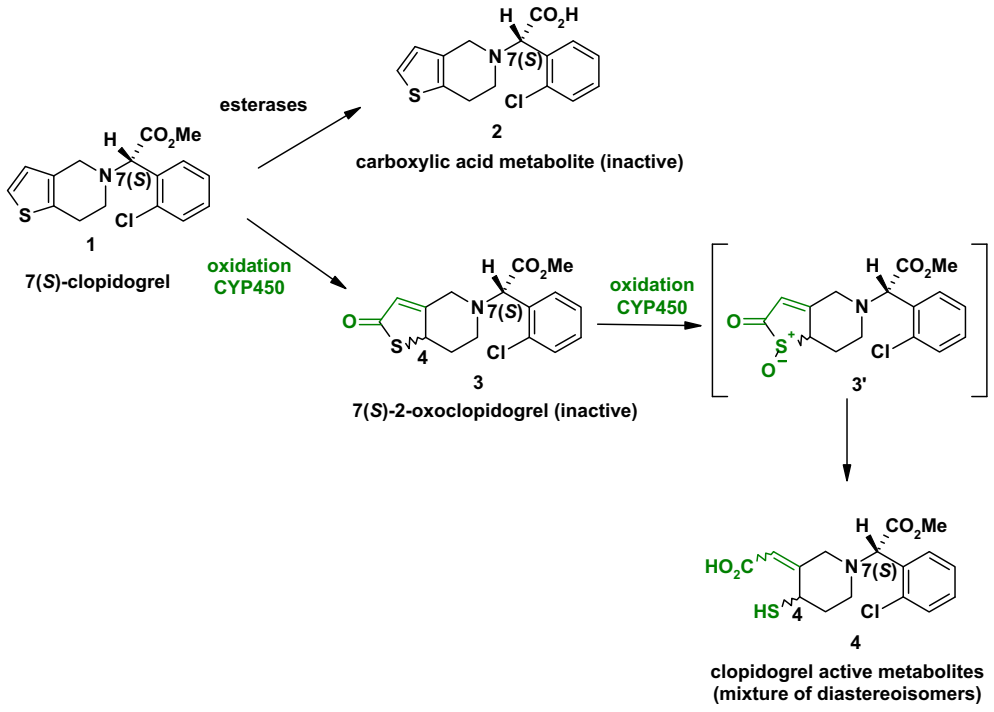
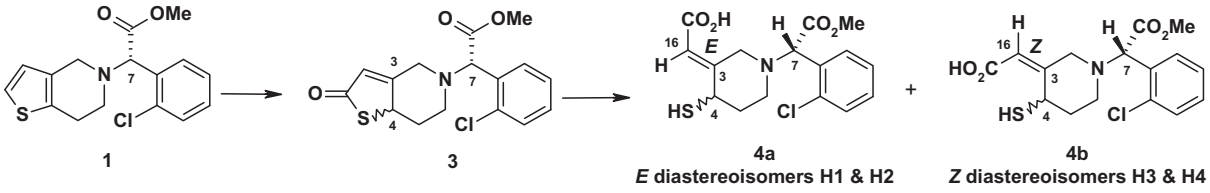


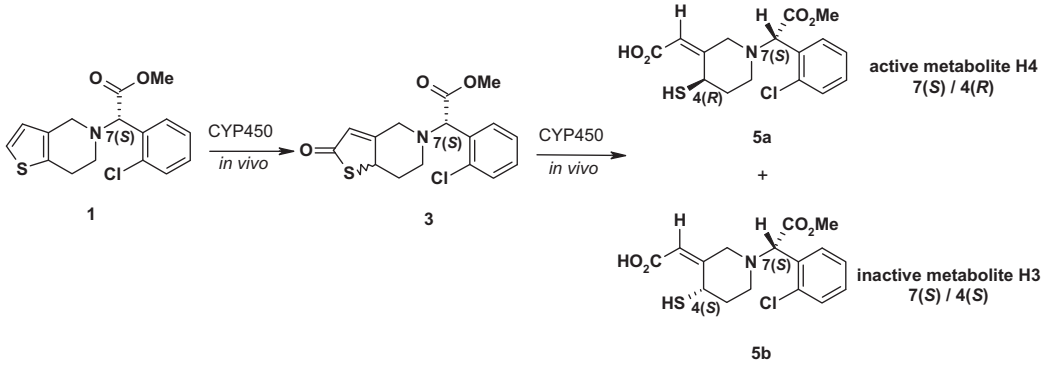
Scheme 1. Metabolic pathway of clopidogrel.



Scheme 2. Active metabolites of clopidogrel: possible set of four stereoisomers generated from clopidogrel **1**.

C4. At the beginning of this project, the absolute configuration at C4 in H4 had not been clearly elucidated. Further structural analysis performed concomitantly with this study, allocated the (*R*) configuration to the C4 centre in active metabolite H4 **5a** (Scheme 3).[5]

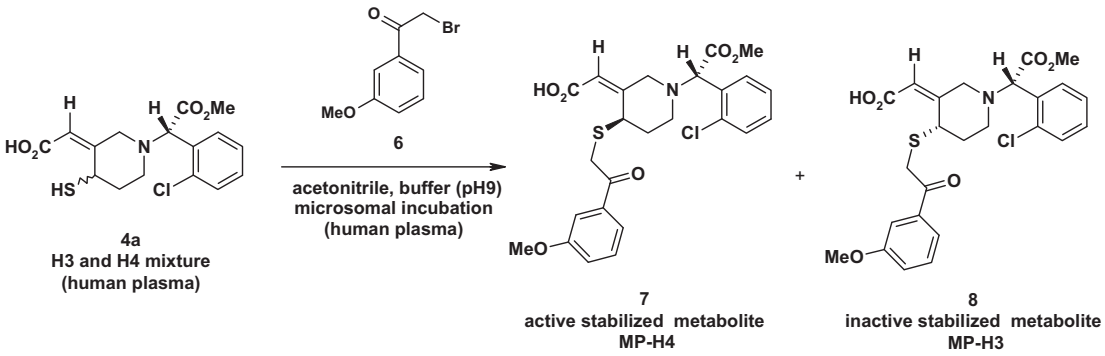
Takahashi et al., showed that direct trapping of metabolites H3 and H4 in human plasma with 2-bromo-3′-methoxy acetophenone **6** as alkylating agent overcomes this instability and allows quantification of the derivatized stable forms of both active metabolite **7** and inactive metabolite **8** in clinical samples (Scheme 4).[6]



Scheme 3. In vivo metabolism: structure of both active metabolite H4 **5a** and inactive metabolite H3 **5b** of **1**.

The presence of a free thiol group makes the clopidogrel metabolites H3 and H4 unstable in human plasma, hampering their quantification in clopidogrel-treated patients.

Derivatized active metabolite **7** has never been synthetized preparatively by chemical means to the best of our knowledge. Previous work relied on microsomal incubation with human liver



Scheme 4. Derivatization of clopidogrel active metabolite with MPBr.

microsomes of **3**.[4] Unfortunately, this process was hampered by low yields, needed extensive purifications and was not suitable for a reliable and efficient preparative scale synthesis. Only small amounts of **7** could be obtained.

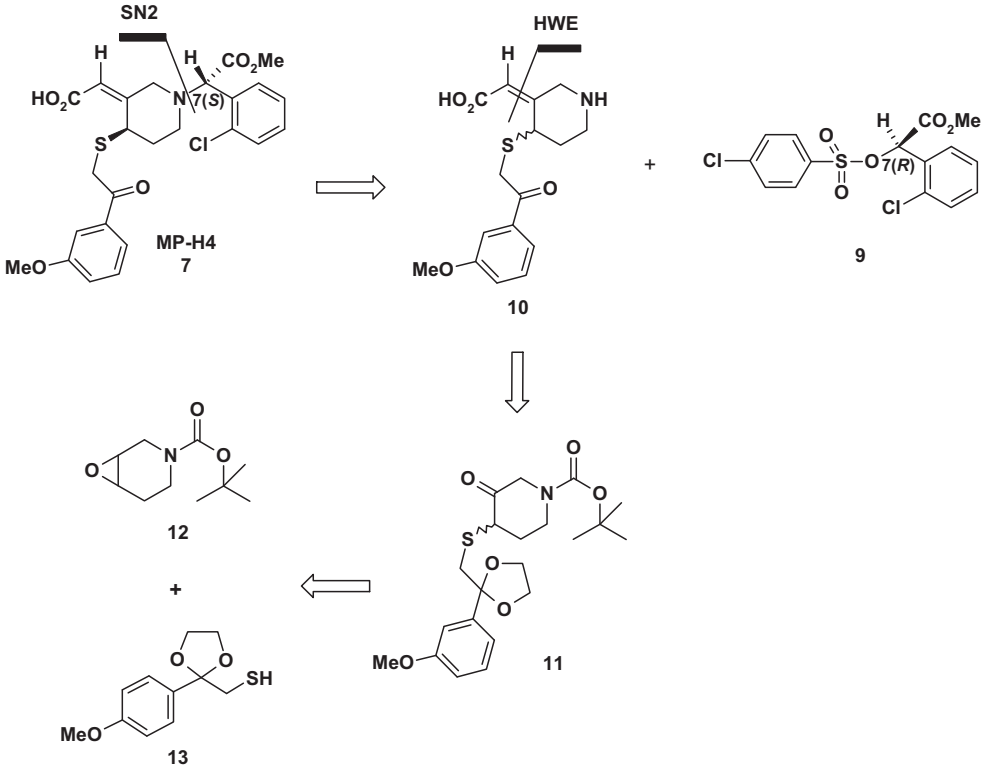
In order to support clinical studies to assess concentrations of MPBr derivative of **5a** in human plasma, larger quantities of **7** were needed, leading us to design a chemical synthesis.

2. Results and discussion

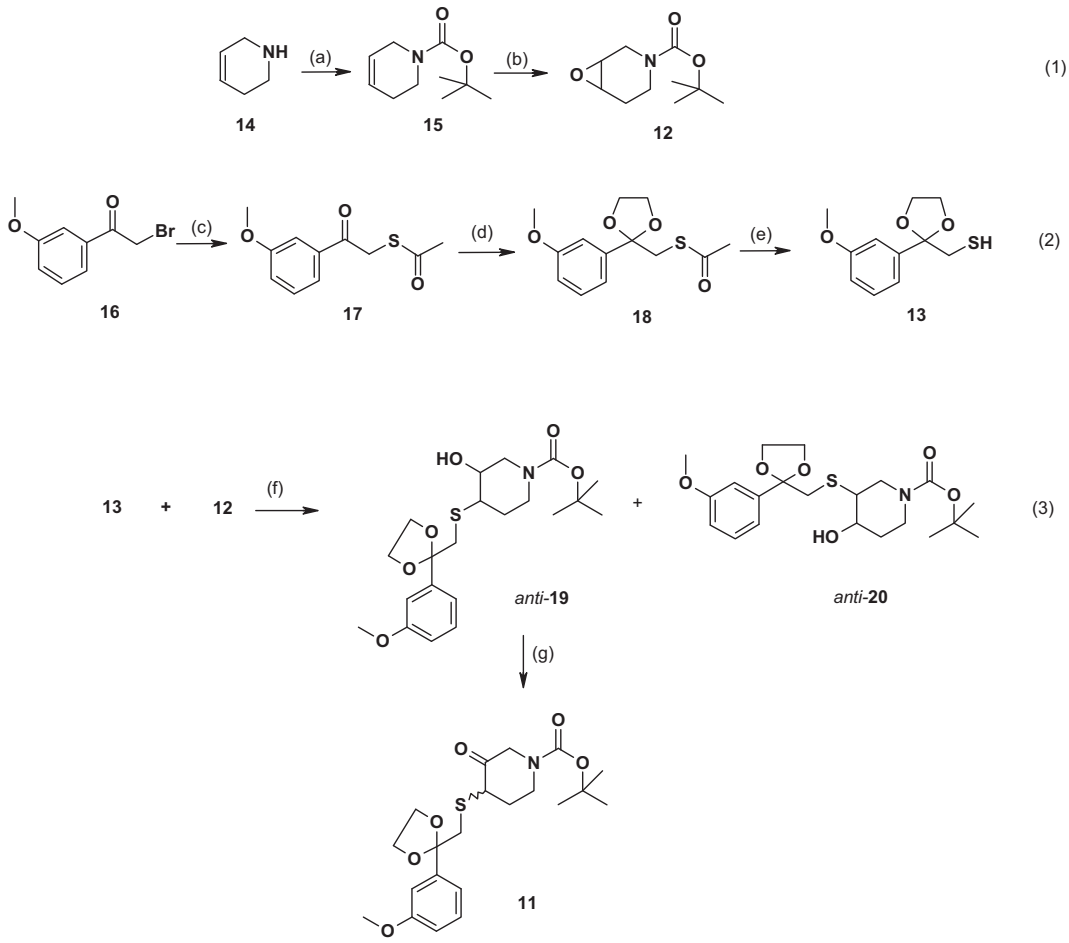
Our retrosynthetic analysis of **7** is illustrated in Scheme 5. The diastereoisomer **7** could be obtained by a S_N2 reaction with stereoinversion, between enantiopure 7(*R*)-chloromandelic acid methyl ester **9** and piperidine derivative **10**, a step based on the current industrial synthesis of clopidogrel.[7] The exocyclic *Z* double bond might then be obtained through a Horner–Wittig–Emmons (HWE) reaction between ketone **11** and a suitable coupling partner, such as an acrylic acid ester derivative. Compound **11** could be prepared by a regioselective opening of BOC-oxopiperidine **12** with a suitable protected thiol **13**.

As shown in Scheme 6, epoxide **12** was prepared from commercially available 1,2,3,6-tetrahydropyridine **14** in two-steps as described in the literature (route 1).[8] Free thiol **13** was obtained from commercially available 2-bromo-3′-methoxy acetophenone (MPBr) **16** in three steps: displacement of the bromide with potassium thioacetate afforded the corresponding *S*-acetate **17**, which was protected as its 1,3-dioxolane, **18** using diethylene glycol and triethyl orthoformate in the presence of catalytic amount of *p*-TSA. Hydrolysis of **18** with 1 M NaOH in MeOH delivered free thiol **13** in up to 75% yield over three steps (route 2).[9]

Regioselective opening of epoxide **12** with 2 equiv of **13** in acetonitrile in the presence of 1 M sodium hydroxide led to the formation of a 90:10 mixture of stereo-regioisomers **19** (major) and **20** (minor) from which desired compound **19** was easily isolated by chromatography on silica gel in 77% yield (route 3).[10] Alcohol **19** was then subjected to a Swern oxidation to afford ketone **11** in up to 72% yield after chromatography on silica gel. This procedure allowed the oxidation of the hydroxyl group without risk to the sulfur moiety, a side reaction, which we observed with the Dess–Martin reagent.



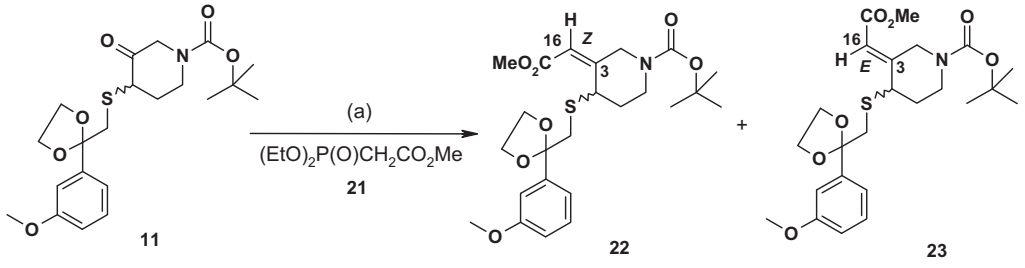
Scheme 5. Retrosynthetic scheme.



Scheme 6. (a) BOC_2O, 1,4-dioxan, 10% aqueous $NaHCO_3$, 16 h (97%); (b) *m*-CPBA, CH_2Cl_2, **12** (71%); (c) KSAc, abs EtOH. in acetone, 2 h at room temperature (100%); (d) diethylene glycol, $HC(OEt)_3$, *p*TSA(5%), 60 °C 24 h (77%); (e) 1 M NaOH, MeOH, 2 h at room temperature (97%); (f) MeCN, 1 M NaOH, 95 °C, 16 h, diastereoisomers not depicted; (g) oxalyl chloride, CH_2Cl_2, DMSO, −78 °C, NEt_3 (72%).

The next step concerned the formation of the C16–C3 exocyclic double bond with *Z* configuration. To this end, we first envisaged introducing the *Z* acrylic acid moiety via a standard HWE reaction on **11** with methyl diethylphosphono acetate **21** (Scheme 7).

To our surprise, the Still–Gennari procedure, commonly employed as a variant of the HWE process to produce *Z*-α,β-unsaturated esters employing NaH as base at 70 °C led to lower yield and unchanged selectivity (entry 2).



Scheme 7. HWE reaction of **11** with **21** (see Table 1).

As expected, under standard conditions, using NaH as base in THF, a mixture of *E*/*Z* isomers was obtained, in which the target *Z* isomer **22** was isolated in 30% yield as the minor component. With the objective of improving the *Z* selectivity and ultimately the yield, we modified the experimental parameters. A variety of different bases and solvents were tested, and the results obtained in the HWE reaction of **11** and **21** are summarized in Table 1. Selectivity and yield were comparable when using either KOH, K_2CO_3 or NaHMDS as base (entry 3, 4 and 5). Replacing THF with toluene and with a high degree of selectivity, in the presence of phosphonate **24**, gave again mainly the *E* isomer **23** (Table 1, entry 6).[11a]

The *Z* selectivity could probably be improved by changing other experimental parameters, such as employing Ando's protocol.[11b] However, the standard HWE conditions applied (Table 1, entry 1) were reliable and efficient enough for our aim to produce the targeted *Z* isomer 22.

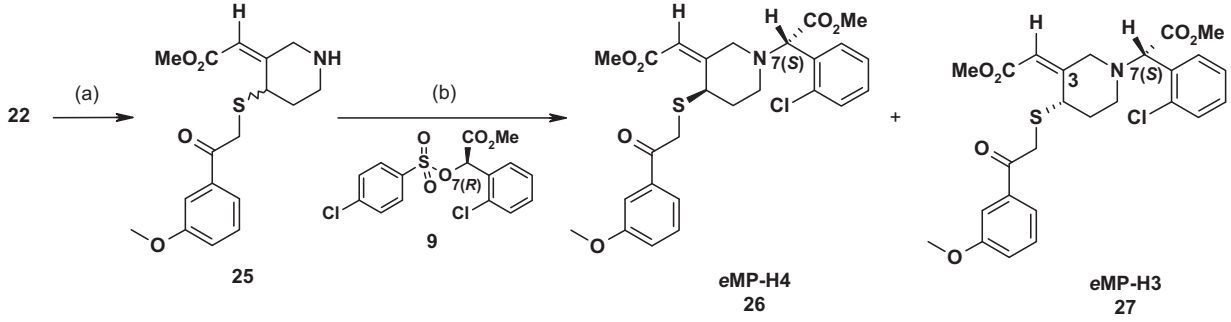
Next, it was necessary to introduce the third stereogenic centre of (*S*) configuration at position 7. For this purpose, the 1,3-dioxolane

Table 1
HWE reaction of **11** with **21**

Entry	Base/solvent/t °C/time	Ratio (**22**)/(**23**)[a]	Yield (**22**)[b]
1	**NaH/THF/0 °C to rt/2.5 h**	**1/2.5**	**30%**
2	NaH/toluene/70 °C/2.5 h	1/3	25%
3	KOH or K_2CO_3/THF/rt/2 h	1/3	Not isolated
4	NaH/THF/−20 °C/8 h	1/3	20%
5	NaHMDS/THF/−78 °C/5 h	1/3	22%
6	KHMDS, 18-crown-6, $(CF_3CH_2O)_2P(O)CH_2CO_2Me$ (**24**)/THF/−78 °C/4 h	1/10	Not isolated

The bold values signifies the best result obtained.
[a] Determined by ^{1}H NMR.
[b] Isolated yield.

and BOC protecting groups in **22** were simultaneously removed using 50% trifluoroacetic acid (TFA) in dichloromethane at room temperature to furnish the secondary amine **25** (Scheme 8). The piperidine nitrogen of **25** was then reacted in a S_N2 reaction with complete stereoinversion with enantiopure 7(*R*)-chloromandelic acid methyl ester **9** in acetonitrile in the presence of potassium bicarbonate.[12] This reaction afforded a 1/1 mixture of diastereoisomers **26** and **27** corresponding to the stabilized methyl ester form of active metabolite **7** and inactive metabolite **8**, respectively.



Scheme 8. (a) TFA/CH_2Cl_2 then saturated aqueous $NaHCO_3$ (93%); (b) **9** (LC purity 99% (by area); 99% e.e.) in CH_3CN then **22**, $KHCO_3$ (2 equiv), 48–72 h.

Considerable effort was needed to separate diastereoisomers **26** and **27** in order to isolate the target compound **26**. We succeeded in isolating both diastereoisomers by preparative chiral chromatography at gram scale (Scheme 9). By applying this method to 3.4 g of 1/1 mixture of **26**/**27** as depicted in Scheme 9 below, 1.3 g of the desired compound **26** were isolated with a chemical purity of 98% and with an optical purity of 99% d.e. (HPLC at 254 nm) .

HPLC-MS data and NMR spectra of stereoisomer **26** were in accordance with the structure and corresponding data obtained for compound **7** generated from incubation of **3** with human microsomes.[4]

The remaining step, the selective hydrolysis of the acrylic methyl ester moiety of **26** to release **7**, was achieved selectively and in acceptable yield, using a 37% HCl aqueous solution, a method previously used in this series.[13]

The desired metabolite **7** was obtained in up to 52% yield after purification on silica gel with 98.6% LC purity and with an optical purity of 99% d.e. (Scheme 10).

3. Conclusion

We have described a convergent synthesis of the stabilized active metabolite **7** of clopidogrel, which was isolated, with chemical purity of 98% and with an optical purity of 99% d.e. Although this eleven steps synthesis was hampered by the low yield of the Horner–Wadsworth–Emmons reaction, the synthesis was robust and efficient enough to prepare sizeable quantities of the stabilized active metabolite **7** of clopidogrel allowing to support a large clinical program.

4. Experimental section

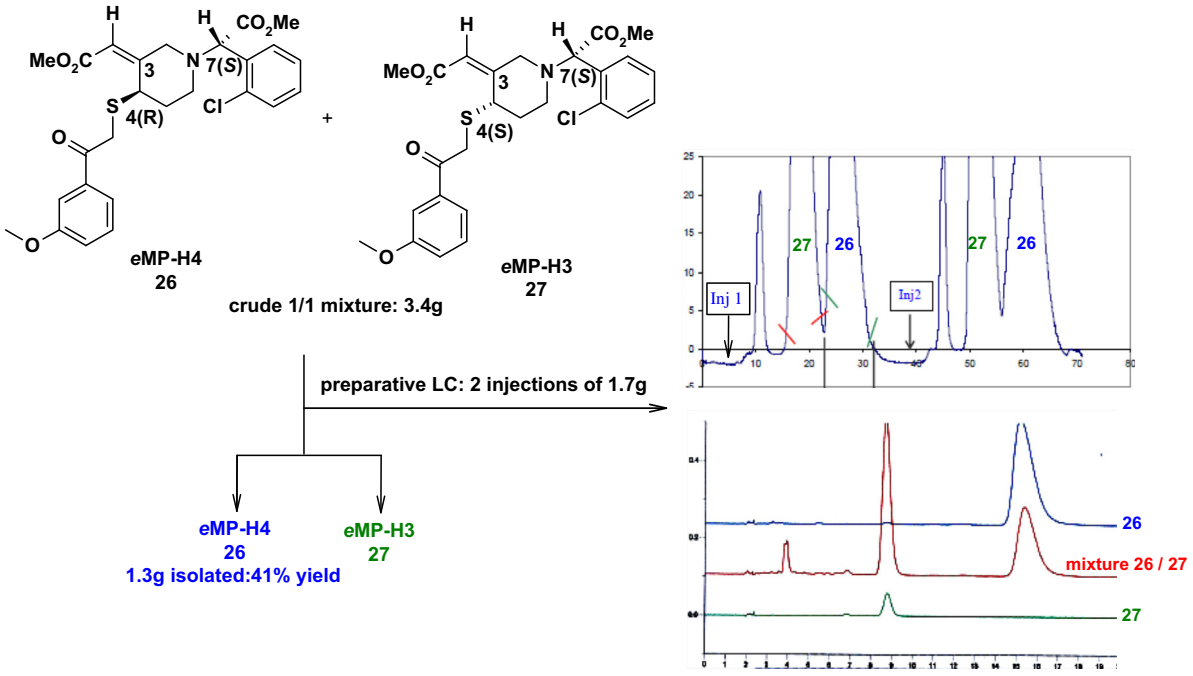
4.1. General

Compounds **14**, **16**, **21** and **24** were purchased from Aldrich and used as received. Compound **9** was obtained from SANOFI, Industrial Affairs, Sisteron, France. Solvents and reagents were purchased from commercial sources. Analytical TLC was performed on Merck (silica gel 60 F_{254}) 5×10 cm plates and visualized with UV light (254 nm). ^{1}H and ^{13}C NMR spectra were recorded from $CDCl_3$ solution with a Bruker DPX 200 (200 MHz) or a Bruker AVANCE 600 (600 MHz) spectrometer. ^{13}C NMR spectra were obtained via DEPTQ experiment. Chemical shifts are given in ppm as δ values with reference to $CDCl_3$. Flash chromatography was conducted on silica gel cartridges with an Armen Spot Prep automated purification system. Chiral preparative liquid chromatography was performed with a chiral preparative Chiralpak® AD column as specified in the experimental procedure. HPLC analyses were recorded on Hitachi Elite LabChrom® (UV 2400) as specified in the experimental procedure. LC-MS/HRMS were recorded with a LC-MS/IT-TOF Shimadzhu system using a Kromasil 150×3.0 mm, C18, 3.5 μm analytical column with a mixture of acetonitrile and 20 mM ammonium acetate (pH 4.6) buffer as mobile phase.

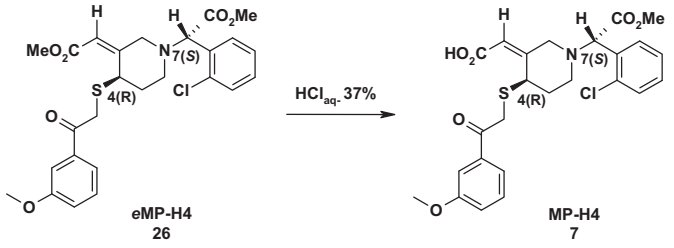
4.2. *tert*-Butyl 7-oxa-4-azabicyclo[4.1.0]heptane-4-carboxylate (12)

1,2,3,6-Tetrahydropyridine **14** (19.7 g, 237 mmol) was dissolved in 1,4-dioxan (20 mL) and 10% aqueous sodium carbonate (20 mL) was added. The solution was cooled to 0 °C and di-*tert*-butyl dicarbonate (54.3 g, 249 mmol) was added in portions to the stirred solution. The ice-bath was removed and the resulting clear yellow solution was stirred overnight at room temperature. The reaction mixture was diluted with brine and ether. The ether phase was separated, and dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to give **15** as a colourless oil used in the next step without purification (42 g, 97%); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.46 (s, 9H), 2.1(br s, 2H), 3.44 (t, J=5.8 Hz, 2H), 3.84(m, 2H), 5.63 (m, 1H), 5.75 (m, 1H).

tert-Butyl-1,2,3,6-tetrahydropyridine-1-carboxylate **15** (30 g, 164 mmol) was then dissolved in dichloromethane (200 mL) and cooled to 0 °C. A solution of *m*-chloroperbenzoic acid (70% w/w, 46.4 g, 188 mmol) in dichloromethane (200 mL) was added dropwise over 30 min. The ice-bath was removed and the resulting colourless mixture stirred overnight. The solution was



Scheme 9. Separation of crude 1/1 mixture of *e*MP-H4 **26**/*e*MP-H3 **27** by preparative chiral LC on Chiralpak AD.



Scheme 10. Hydrolysis of ester **26** to free acid **7**.

filtered to remove salts and washed with 5% aqueous potassium carbonate, brine and three times with saturated aqueous sodium thiosulfite. The organic layer was separated, dried over anhydrous sodium sulfate, filtered and evaporated in vacuo to give **12** as a clear yellow oil. The crude product was subjected to flash chromatography (SiO_2, pentane/diethylether 70/30) to afford the pure title compound **12** (23 g, 71%). R_f=0.31 (SiO_2, pentane/diethylether 7/3); ^{1}H NMR (200 MHz, $CDC1_3$) δ: 1.32 (s, 9H), 1.67–2.0 (m, 2H), 2.9–3.35 (m, 4H), 3.51–3.78 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 154.8, 79.8, 50.6, 50.1 (br), 42.8, 41.9, 37.9, 36.4, 28.4, 24.4.

4.3. 2-(Acetylsulfanyl)-1-(3-methoxyphenyl)ethan-1-one (10)[14]

To a suspension of potassium thioacetate (18.7 g, 163 mmol) in absolute ethanol (100 mL) was added dropwise a solution of 3′-methoxyphenacyl bromide (MP-Br) **16** (25 g, 109 mmol) in acetone (80 mL). The reaction mixture was then stirred for 2 h at room temperature. The dark yellow solution was filtered to remove KBr and the filtrate evaporated to dryness. The residue was dissolved in ether and the solution washed with water dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to afford **17** in quantitative yield (25 g). R_f=0.63 (SiO_2, pentane/diethylether 7/3); ^{1}H NMR (200 MHz, $CDC1_3$) δ: 2.21 (s, 3H), 3.65 (s, 3H), 4.20 (s, 2H), 6.93–7.00 (m, 1H), 7.16–7.40 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) 197.9, 193.9, 157.8, 135.7, 128.6, 121.9, 119.7, 110.2, 52.1, 33.5, 29.7.

4.4. 2-(3-Methoxyphenyl)-1,3-dioxolanylmethanethiol (13)

Compound **17** was dissolved in diethylene glycol (105 mL, 2 mol) and triethyl orthoformate (145 mL, 0.9 mol) was added, followed by the addition of a catalytic amount of *p*-toluenesulfonic acid (1.1 g, 6.3 mmol). The reaction mixture was heated at 60 °C and stirred for 24 h. The greenish solution was cooled to room temperature and the solvents concentrated in vacuo. The oily residue was diluted with brine and extracted with ether. The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to yield 41 g of a greenish oil. Purification by chromatography on silica gel (eluent pentane/diethylether 6/4) afforded 25.6 g (77% yield) of 1-((2-(3-methoxyphenyl)-1,3-dioxolan-2-yl)sulfanyl) ethan-1-one **18**: R_f=0.42 (SiO_2, pentane/diethylether 7/3); GC–MS (CI): M+H$^+$ at 268.9 (12.5%) with fragments at 87.0 (100%), 179.1 (25%) and 209.0 (10%), ^{1}H NMR (200 MHz, $CDC1_3$) δ: 2.3 (s, 3H), 3.45 (s, 2H), 3.82 (m, 5H, $-OCH_3-$, $-CH_2O-$), 4.07 (m, 2H, $-CH_2O-$), 6.83–6.87 (m, 1H), 7.07–7.35 (m, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 197.9, 193.9, 157.8, 135.7, 128.6, 121.9, 119.7, 110.2, 52.1, 33.5, 29.7.

Compound **18** was dissolved in methanol (200 mL) and 1 M sodium hydroxide (105 mL, 105 mmol) was added. The reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated in vacuo and brine was added, followed by extraction with diethylether. The organic layer was dried on anhydrous sodium sulfate and evaporated to yield 21 g (97%) of the unstable title compound **13**, used in the next step without purification. R_f=0.59 (SiO_2, pentane/diethylether 7/3); GC–MS (CI): M+H$^+$ at 227 (100%) with fragments at 193.3 (37%), 183.3 (70%), 149.2 (25%), 119.2 (20%).

4.5. *tert*-Butyl 3-hydroxy-4-[2-((3-methoxyphenyl)-1,3-dioxolan-2-yl]methylsulfanyl)piperidine-1-carboxylate (19)

To a stirred solution of epoxide **12** (7.3 g, 37 mmol) in acetonitrile (150 mL) was added dropwise a solution of **13** (16.6 g,

73.3 mmol) in acetonitrile (100 mL) followed by 1 M aqueous sodium hydroxide solution (74 mL, 74 mmol). The mixture was heated at 95 °C for 24 h. The progress of the reaction was monitored by TLC (SiO_2, pentane/ether 6/4). The solvent was evaporated in vacuo and brine was added, followed by extraction with diethylether. The organic layers were dried over anhydrous sodium sulfate, filtered and evaporated to afford a crude mixture of regioisomers **19** and **20**. Purification by flash column chromatography on silica gel (eluent pentane/diethylether 6/4) gave 12 g of the target compound **19** (77% yield) as a clear yellow gum. R_f=0.35 (SiO_2, pentane/diethylether 6/4, R_f **20**=0.23); ^{1}H NMR (600 MHz, $CDC1_3$) δ: 1.45 (s, 9H), 1.5 (m, 1H, −CHax−), 1.95 (m, 1H, −CHeq−), 2.53 (m, 1H, S−CHax−), 2.60 (m, 1H, N−CHax−), 2.71 (br s, 1H, N−CHax−), 3.07 (d, J=15 Hz, 1H, −S−CH_2−), 3.19 (d, J=14.4 Hz, 1H, −S−CH_2−), 3.40 (br s, 1H, −CHax−OH), 3.81 (s, 3H, −OCH_3−), 3.88 (m, 2H, −CH_2O−), 4.05 (br s, 1H, −N−CHeq−), 4.15 (m, 2H, −CH_2O−), 4.28 (m, 1H, −N−CHeq−), 6.86 (m, 1H, Ar), 7.01 (m, 1H, Ar), 7.04 (m, 1H, Ar), 7.25 (m, 1H, Ar); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.6, 154.6, 142.8, 129.4, 118.0, 114.0, 111.4, 109.2, 79.9, 71.1, 65.5, 65.3, 55.3, 52.6, 41.1, 31.5, 28.4; LC-MS: 97% LC purity (by area); ESMS m/z (%): 448 (100) [M+Na^+]; 464 (34) [M+K^+]; 326(35) [M−BOC+H^+]; 308(38) [326−H_2O]]; 264(85) [308−C_2H_4O].

4.6. *tert*-Butyl-4-[2-((3-methoxyphenyl)-1,3-dioxolan-2-yl] methylsulfanyl)-3-oxopiperidine-1-carboxylate (11)

Under a nitrogen atmosphere, a solution of dimethylsulphoxide (6 mL, 84 mmol) in dichloromethane (8 mL) was added to a stirred solution of oxalyl chloride (3.7 mL, 42.3 mmol) in dichloromethane (150 mL) at −78 °C; The reaction mixture was stirred for 45 min before **19** (12 g, 28 mmol) in dichloromethane (100 mL) was slowly added such that the temperature did not rise above −65 °C. The mixture was stirred for a further 45 min before triethylamine (20 mL, 141 mmol) was added. This reaction mixture was stirred for an additional 1 h and poured onto a mixture of ice and saturated brine followed by extraction with diethylether. The organic layers were combined, dried over anhydrous sodium sulfate, filtered and concentrated to give an orange oil. Purification by flash column chromatography on silica gel using pentane/diethylether 70/30 as eluent afforded 8.5 g of **11** (72%) as an unstable yellow oil stored at −20 °C. R_f=0.5 (SiO_2, pentane/diethylether 1/1); ^{1}H NMR (600 MHz, $CDC1_3$) δ: 1.47 (s, 9H), 2.03 (m, 1H, −CHeq−), 2.27 (m, 1H, −CHax−), 2.95 (d, J=18.6 Hz, 1H, −S−CH_2−), 3.07 (m, 1H, −S−CH_2−), 3.39 (br s, 1H, N−CHax−), 3.44 (m, 1H, −S−CHax−), 3.72 (br s, 1H, −N−CHeq−), 3.82 (s, 3H, −OCH_3), 3.85 (m, 2H, −CH_2−O−), 4.08−4.14 two signals mixed up (m, 2H, −O−CH_2−) and (d, J=22 Hz, 1H, CO−CH_2−N−), 4.24 (d, J=22 Hz, 1H, −CO−CH_2−N−), 6.84 (m, 1H, Ar), 7.01(m, 1H, Ar), 7.04 (m, 1H, Ar), 7.25 (m, 1H, Ar); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.5, 154.2, 142.8, 129.3, 118.2, 114.1, 111.4, 109.2, 80.5, 65.4, 65.3, 55.3, 48.3, 40.8, 29.7, 28.3.

4.7. *tert*-Butyl-3(*Z*)-3-((2-methoxy-2-oxoethylidene)-4-[2-(3-methoxyphenyl)-1,3-dioxolan-2-yl]methylsulfanyl) piperidine-1-carboxylate (22)

Under a nitrogen atmosphere, a suspension of NaH (60% w/w in mineral oil, 1 g, 25 mmol) in anhydrous THF (30 mL) was treated dropwise with methyl diethylphosphono acetate **21** (4.4 mL, 23 mmol) between 0 °C and −5 °C, and the mixture was stirred for 1 h at 0 °C. A solution of ketone **11** (8 g, 19 mmol) in THF (40 mL) was then added dropwise and the reaction mixture allowed to stir at room temperature until TLC (SiO_2 pentane/diethylether 6/4) showed that all the ketone **11** was consumed (2 h). The mixture was concentrated in vacuo and the residue was diluted with CH_2Cl_2 and brine.

The organic layer was separated, dried over sodium sulfate, filtered and evaporated to afford 10 g of a clear yellow oil (1:3 mixture of *E* **23** and *Z* **22** isomers). Purification by chromatography on silica gel using pentane/diethylether 60/40 allowed the isolation of 2.3 g of the target *Z* isomer **22** (25%). R_f (*Z*, **22**)=0.6, R_f (*E*, **23**)=0.5 (SiO_2, pentane/diethylether 1/1); ^{1}H NMR (600 MHz, $CDC1_3$), **22**(*Z*) δ: 1.44 (s, 9H), 1.90 (m, 1H, −CHeq−), 1.99 (br s, 1H, −CHax−), 2.93 (d, J=18 Hz, 1H, −S−CH_2−), 3.16 (d, J=18 Hz, 1H, −S−CH_2−), 3.18 (br s, 1H, −N−CHax−), 3.69 (s, 3H, −CO_2CH_3), 3.81 (s, 3H, −OCH_3), 3.84 (d, J=18 Hz, 2H, −CH_2O−), 3.96 (br s, 1H, −N−CHeq−), 4.11 (m, 2H, −OCH_2−), 5.28 (s, 1H, −S−CH−), 5.73 (s, 1H, −CH=), 6.83 (m, 1H, Ar), 7.04 (m, 1H, Ar), 7.07 (m, 1H, Ar), 7.24 (m, 1H, Ar); ^{13}C NMR (150 MHz, $CDCl_3$), **22**(*Z*) δ: 166.5, 159.6, 154.4, 143.3, 129.2, 118.2, 113.9, 111.4, 109.2, 80.0, 65.3, 55.1, 51.1, 41.1, 40.5, 32.2, 28.6. HRMS (ESI$^+$), **22**(*Z*): m/z calcd for $C_{24}H_{33}NO_7S$ [M+Na]$^+$: 502.1870; found, 502.1820.

^{1}H NMR (600 MHz, $CDC1_3$), **23**(*E*) δ: 1.45 (s, 9H), 1.86 (m, 1H, −CHeq−), 2.08 (br s, 1H, −CHax−), 2.84 (q, J=12 Hz, 2H, −S−CH_2−), 3.26 (br s, 1H, −N−CHax−), 3.61 (m, 1H, −CHeq−), 3.73 (s, 3H, −CO_2CH_3), 3.82 (s, 3H, −OCH_3), 3.84−3.86 (m, 3H), 3.94 (d, J=18 Hz, 1H), 4.07−4.14 (m, 2H, −OCH_2−), 5.48 (d, J=18 Hz, 1H, −S−CH−), 5.67 (s, 1H, −CH=), 6.85 (m, 1H, Ar), 7.02 (s, 1H, Ar), 7.05 (m, 1H, Ar), 7.28 (m, 1H, Ar); ^{13}C NMR (150 MHz, $CDCl_3$), **23**(*E*) δ: 166.6, 159.6, 154.3, 143.2, 129.3, 118.1, 116.3, 113.9, 111.3, 109.5, 79.8, 65.3, 65.1, 55.2, 51.3, 47.6, 41.1, 40.4, 31.8, 28.4; HRMS (ESI$^+$), **23**(*E*): m/z calcd for $C_{24}H_{33}NO_7S$ [M+Na]$^+$: 502.1870; found, 502.1829.

4.8. Methyl 7(*S*)-7-((2-chlorophenyl)-2-(3(*Z*)-3-methoxy-2-oxoethylidene)-4-(2-(3-methoxyphenyl)-2-oxoethyl)sulfanyl piperidin-1-yl)acetate mixture of diastereoisomers as acrylic methyl ester form eMp-H4 (26) and eMp-H3 (27)

Under a nitrogen atmosphere, a stirred solution of compound **22** (2.3 g, 5 mmol) in dichloromethane (30 mL) cooled at 0 °C was treated dropwise with a 50% solution of trifluoroacetic acid (7.4 mL, 96 mmol) in dichloromethane (15 mL). The clear yellow reaction mixture was then stirred for 24 h at room temperature. The progress of the reaction was monitored by TLC on silica gel using CH_2Cl_2/MeOH/NH_4OH (9/1/0.1) as eluent. The mixture was concentrated in vacuo and the residue diluted with water, basified to pH 8 with a saturated aqueous solution of $NaHCO_3$ and extracted several times with dichloromethane. The organic layers were combined, dried over anhydrous sodium sulfate and the solvent evaporated to afford 1.5 g of the unstable crude amine **25** (93%) used in the next step without purification. ES-MS m/z (%): 336 (100) [M+H^+]; 358 (10) [M+Na^+]; 374 (5) [M+K^+]; HRMS: m/z calcd for $C_{17}H_{21}NO_4S$ [M+H]$^+$: 336.1264; found, 336.1287.

A 40% solution of 7-(*R*)-chloromandelic acid methyl ester (7.35 g, 7.8 mmol) **9** in dichloromethane was concentrated by solvent removal under a stream of nitrogen. The clear residual oil was dissolved in acetonitrile (15 mL) and a solution of compound **25** (1.5 g, 4.5 mmol) in acetonitrile (3 mL) was added dropwise, followed by the addition of $KHCO_3$ (0.9 g, 9 mmol), in one portion. The reaction mixture was stirred for 72 h, under nitrogen atmosphere at room temperature, and the resultant yellow precipitate was filtered off, and washed with acetonitrile and ethyl acetate. The combined washings and filtrate were evaporated to afford 4 g of a crude orange oil. Purification by chromatography on silica gel (pentane/diethylether or heptane/ethyl acetate, 70/30) led to 2 g of a mixture of both diastereoisomers *e*MP-H4 **26** and *e*MP-H3 **27**.

The diastereoisomers **26** and **27** were then separated and isolated by preparative chiral chromatography using a Chiralpak AD column (Di 100 mm, dp20 μm); eluent EtOH/MeOH/Heptane (15/15/70); flow rate: 400 mL/min; detector: 254 nm. In this way 1.3 g of the desired diastereoisomer **26** (39%) and 1.4 g (41%) of

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diastereoisomer **27** were isolated as a clear yellow oil and as a white solid, respectively.

Chiral HPLC analysis Chiralpak AD-H 5 μm (250×4.6 mm); eluent EtOH/MeOH/Heptane (15/15/70); flow rate: 1.5 mL/min; detector: 254 nm; acrylic ester *e*MP-H3 **27** retention factor k_1'=4; acrylic ester *e*MP-H4 **26** k_2'=7.6. ^{1}H NMR (600 MHz, $CDCl_3$), *e*MP-H4 **26** δ: 1.91 (d, *J*=14.6 Hz, 1H, −CHeq−), 2.17 (m, 1H, −CHax−), 2.61−2.74 (m, 2H, −CHax−, −CHeq−), 3.12 (d, *J*=12.3 Hz, 1H, $-CH_2-$), 3.60 (s, 3H, $-CO_2CH_3$), 3.64 (d, *J*=12.1 Hz, 1H, $-CH_2-$), 3.71 (s, 3H, $-CO_2CH_3$), 3.86 (s, 3H, $-OCH_3$), 3.96 (d, *J*=15.8 Hz, 1H, $-CH_2-$), 4.07 (d, *J*=15.8 Hz, 1H, $-CH_2-$), 4.80 (s, 1H, $-CH-CO_2CH_3-$), 5.29 (m, 1H, −S−CH−), 5.72 (s, 1H, −CH═), 7.11 (m, 1H, Ar), 7.25−7.30 (br m, 2H, Ar), 7.35−7.40 (m, 2H, Ar), 7.44−7.49 (m, 2H, Ar), 7.59 (m, 1H, Ar); ^{13}C NMR (150 MHz, $CDCl_3$), *e*MP-H4 **26** δ: 194.7, 171.0, 166.5, 159.9, 154.4, 137.3, 134.9, 1333.3, 129.8, 129.6, 127.2, 121.1, 120.0, 116.6, 112.8, 68.0, 58.4, 55.5, 54.5, 52.3, 51.3, 46.1, 40.0, 38.8, 31.0; HRMS (ESI$^+$), *e*MP-H4 **26**: *m*/*z* calcd for $C_{26}H_{28}NO_6SCl$ $[M+Na]^+$: 540.1218.; found, 540.1250.

^{1}H NMR (600 MHz, $CDC1_3$), *e*MP-H3 **27** δ: 1.97 (d, *J*=14.4 Hz, 1H, −CHeq−), 2.25 (m, 1H, −CHax−), 2.75−2.89 (m, 2H, CHax−, −CHeq−), 2.91 (d, *J*=12 Hz, 1H, $-CH_2-$), 3.55 (d, *J*=12 Hz, 1H, $-CH_2-$), 3.59 (s, 3H, $-CO_2CH_3$), 3.70 (s, 3H, $-CO_2CH_3$), 3.85 (s, 3H, $-OCH_3$), 3.94 (d, *J*=15.8 Hz, 1H, $-CH_2-$), 4.06 (d, *J*=15.6 Hz, 1H, $-CH_2-$), 4.80 (s, 1H, $-CH-CO_2CH_3-$), 5.28 (m, 1H, −S−CH−), 5.60 (s, 1H, −CH═), 7.10 (m, 1H, Ar), 7.25−7.30 (br m, 2H, Ar), 7.35 (m, 1H, Ar), 7.41 (m, 1H, Ar), 7.47 (m, 1H, Ar), 7.61 (m, 1H, Ar); ^{13}C NMR (150 MHz, $CDCl_3$), *e*MP-H3 **27** δ: 194.4, 171.1, 166.4, 159.9, 154.7, 137.3, 134.7, 133.3, 129.9, 129.3, 127.2, 121.2, 119.9, 116.6, 112.8, 67.9, 55.5, 54.3, 52.2, 51.3, 46.6, 39.8, 38.8, 31.2; HRMS (ESI$^+$), *e*MP-H3 **27**: *m*/*z* calcd for $C_{26}H_{28}NO_6SCl$ $[M+H]^+$: 518.1399; found, 518.1413.

4.9. 2-(((3*Z*,4*R*)-1(7*S*-1-(2-Chlorophenyl)-2-methoxy-2-oxoethyl)-4-(2-(3-methoxyphenyl)-2-oxoethyl)sulfanyl)piperidin-3-ylidene)acetic acid, stabilized active metabolite MP-H4 (7)

Acrylic methyl ester *e*MP-H4 **26** (1 g, 2 mmol) isolated by chiral preparative chromatography as described in Section 4.8, was dissolved in 37% aqueous HCl (50 mL) at room temperature. The reaction mixture was then stirred at 40−45 °C for 36 h. The greenish solution was allowed to reach room temperature and cooled at 0 °C. A saturated solution of $NaHCO_3$ was carefully added dropwise to adjust the pH to 7−8 and the reaction mixture extracted with ethyl acetate and dichloromethane.

The organic layers were combined, dried over anhydrous sodium sulfate, filtered and the solvent evaporated to afford a green oil. Purification by chromatography on silica gel (CH_2Cl_2/MeOH, 94/6) afforded 0.5 g of an off-white solid corresponding to the expected stabilized active metabolite MP-H4 **7** of clopidogrel (52% yield).

Chiral HPLC analysis: Chiralpak AD-H 5 μm (250×4.6 mm) eluent EtOH/Heptane 30/70; 40 °C; flow rate: 1 mL/min; detector: 254 nm; diastereoisomeric purity for MP-H4 **7** (4*R*, 7*S*): 99%; HPLC analysis: Shim-pack XR-ODS II, 2.2 μm (75×2 mm) eluent: ammonium acetate buffer+acetonitrile, gradient; 35 °C; flow rate: 0.5 mL/min; detector: 254 nm; HPLC purity (by area): 98.6%.

^{1}H NMR (600 MHz, $CDC1_3$) δ: 1.93 (d, *J*=14.4 Hz, 1H, −CHeq−), 2.15 (br m, 1H, −CHax−), 2.64−2.73 (m, 2H, −CHax−, −CHeq−), 3.15 (d, *J*=12 Hz, 1H, $-CH_2-$), 3.66 (d, *J*=12 Hz, 1H, $-CH_2-$), 3.71 (s, 3H, $-CO_2CH_3$), 3.82 (s, 3H, $-OCH_3$), 3.93 (d, *J*=15.8 Hz, 1H, $-CH_2-$), 4.06 (d, *J*=15.6 Hz, 1H, $-CH_2-$), 4.81 (s, 1H, $-CH-CO_2CH_3-$), 5.21 (m, 1H, −S−CH−), 5.74 (s, 1H, −CH═), 7.06 (m, 1H, Ar), 7.27−7.35 (m, 3H, Ar), 7.40−7.48 (m, 3H, Ar), 7.61 (m, 1H, Ar); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 194.5, 171.1, 159.8, 136.9, 134.8, 133.3, 130.1, 129.4, 127.3, 121.1, 119.9, 116.4, 112.7, 67.9, 55.4, 54.7, 52.2, 46.0, 39.8, 38.6, 31.0; HRMS (ESI$^+$): *m*/*z* calcd for $C_{25}H_{26}NO_6SCl$ $[M+H]^+$: 504.1242; found, 504.1148.

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