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# Chemo-enzymatic approach to the synthesis of the antithrombotic clopidogrel

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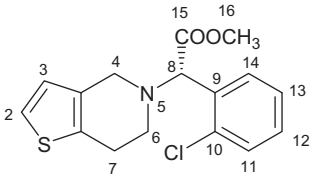
ABSTRACT

The (*S*)-2-chlorophenylglycine moiety is well recognized in the structure of (*S*)-clopidogrel, a known antithrombotic drug. We prepared an enantiomerically pure chiral building block via an enzyme-catalyzed resolution of (*RS*)-*N*-Boc-2-chlorophenylglycine methylester. The best results were obtained by means of an immobilized subtilisin, the cross-linked enzyme aggregate (Alcalase-CLEA®). The high enantiomeric excess of the synthon obtained remained the same over the course of clopidogrel synthesis; the simplicity of the process makes this pathway suitable for large-scale preparation.

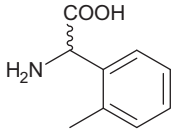


## 1. Introduction

Clopidogrel **1** is an antiaggregatory and antithrombotic drug administered for the reduction of atherosclerotic events including myocardial infarction, ischemic stroke, and peripheral vascular disease, widely used in combination with aspirin after placement of intravascular stents.[1–4]




**1**




**2**

Among the two possible stereoisomers, only the (*S*)-enantiomer is suitable for pharmaceutical application, since the (*R*)-enantiomer is devoid of antithrombotic activity and causes convulsions in animal experiments.[5] 2-Chlorophenylglycine **2**, an unnatural amino-acid commercially available as a racemic mixture, is a valuable intermediate for the synthesis of clopidogrel **1**, with the substituents at the stereogenic center of the two molecules being strictly related.

Several syntheses of (*S*)-**1** start from the resolution of (*RS*)-2-chlorophenylglycine, or its esters, via the formation of diastereomeric salts with tartaric[6] or camphor sulfonic acid[7] followed by fractional crystallization.

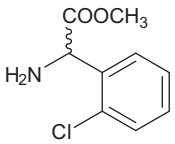
Other, economically less convenient preparations, since 50% of the final product has to be discarded, delay the resolution to the last step, that is, crystallization of (*RS*)-clopidogrel with (1*R*)-camphor sulfonic acid,[8] or of its precursor with (2*R*,3*R*)-tartaric acid.[9]

The presence of a benzylic proton at the α-position makes arylglycines racemization prone:[10] the mild conditions and the stereoselectivity of enzymatic transformations prompted us to attempt a biocatalytic approach in order to obtain enantiomerically pure 2-chlorophenylglycin with the required configuration. While numerous examples of successful enzyme-catalyzed phenylglycines resolutions have already been reported in the literature,[11–15] only a few cases of 2-chlorophenylglycines are described. D-Phenylglycine and several analogs, variously substituted on the aromatic ring, 2-chlorophenylglycine included, useful starting material for semisynthetic penicillins and cephalosporins, have been already prepared by employing D-hydantoinase.[16] (*RS*)-2-Chlorophenylglycine **2** has already been successfully resolved by Fadnavis et al. by employing immobilized penicillin G acylase.[17]

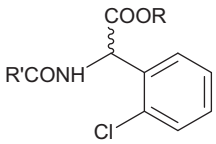
## 2. Results and discussion

### 2.1. Enzyme-catalyzed resolution of (*RS*)-2 -chlorophenylglycine 2

First, we planned to explore the possible biocatalyzed transformations of either the carboxylic or the amino group, that is, the enzyme-catalyzed hydrolysis or synthesis of ester **3** and amides **4**.




**3**




**4**

**a** R = $CH_3$, R '= $CH_3$
**b** R = $CH_3$, R' = $CF_3$
**c** R = H, R' = $CH_3$
**d** R = H, R' = $CF_3$

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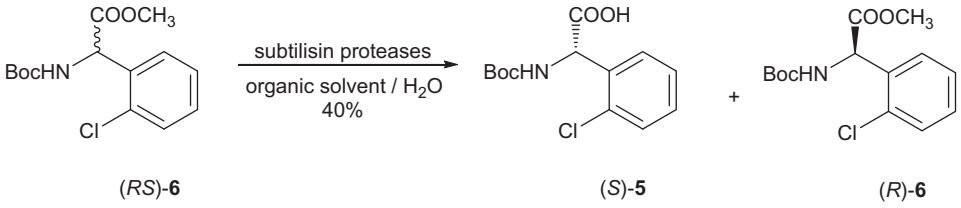
**Table 1**
Enzymatic hydrolysis of (*RS*)-**3**

| Entry | Enzyme | Solvent | Reaction time (h) | Conversion (%)[a] | ee (%)[a] (*S*)-acid **2**[b] |
|---|---|---|---|---|---|
| 1 | CAL B | pH 7.5 buffer | 12 | 44 | 11 |
| 2 | CAL B | pH 7.5 buffer, $BMIMBF_4$ | 10 | 50 | 30 |
| 3 | PPL | pH 7.5 buffer | 8 | 45 | 24 |
| 4 | PFL | pH 7.5 buffer | 12 | 45 | 10 |
| 5 | CCL | pH 7.5 buffer | 12 | 45 | 0 |
| 6 | Papain | pH 7.5 buffer | 12 | 39 | 0 |
| 7 | Chymotripsin | pH 7.5 buffer | 4 | 42 | 67 |
| 8 | Chymotripsin | pH 7.5 buffer/20% butanol | 27 | 46 | 16 |
| 9 | Chymotripsin | pH 7.5 buffer, $BMIMBF_4$ | 8 | 32 | 12 |
| 10 | Protease from *Bacillus licheniformis* | pH 7.5 buffer | 12 | 70 | 27[c] |
| 11 | Protease Carlsberg | pH 7.5 buffer | 12 | 70 | 23[c] |

[a] Calculated from HPLC.
[b] The configuration of the acid obtained was assigned by comparison with HPLC of enantiomerically pure (*S*)-**2** and (*S*)-**3** (see below).
[c] (*S*)-Configuration belongs to unreacted ester **3**.




(*RS*)-**6** (*S*)-**5** (*R*)-**6**

**Scheme 1.** Subtilisin proteases-catalyzed hydrolysis of (*RS*)-**6**.

**Table 2**
Enzymatic hydrolysis of (*RS*)-**6**

| Entry | Enzyme | Solvent | Reaction time (h) | Conversion (%)[a] | ee (%)[b](*S*)-acid **5**[c] |
|---|---|---|---|---|---|
| 1 | PPL | pH 7.5 buffer/20% hexane | 240 | 10 | 54 |
| 2 | Protease from *Bacillus licheniformis* | pH 7.5 buffer/TBME 2:1 | 65 | 40 | >98 |
| 3 | Protease Carlsberg | pH 7.5 buffer/TBME 2:1 | 63 | 40 | >98 |
| 4 | Alcalase CLEA | pH 8 buffer/$CH_3CN$ 10:1 | 15 | 40 | 79 |
| 5 | Alcalase CLEA | pH 8 buffer/DMF 10:1 | 16 | 40 | 65 |
| 6 | Alcalase CLEA | pH 8 buffer/THF 10:1 | 14 | 40 | 98 |

[a] Determined by HPLC on a C18 stationary phase.
[b] Determined by HPLC on a chiral stationary phase after Boc removal.
[c] The configuration of acid **5** was assigned, after esterification to **6**, by comparison with the reported $[\alpha]_D$ value.[21]

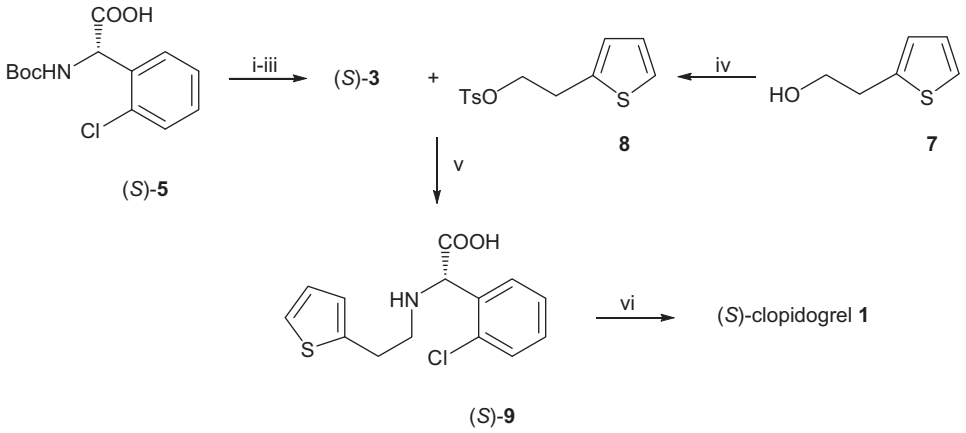
Our screenings started from the lipases, while bearing in mind their properties of stability, selectivity, and commercial availability for large-scale production. Nevertheless, despite their well-documented selectivity on a wide class of substrates, aminoacids included, only low ee (0–25%) were obtained after 2-chlorophenyl glycine methylester **3** hydrolysis, employing lipases from different origins (lipase from porcine pancreas, PPL, from *Pseudomonas fluorescens*, PFL, from *Candida cylindracea*, CCL, from *Candida antarctica*, CAL B) (Table 1, entries 1, 3–5). The addition of an ionic liquid, 1-butyl-3-methylimidazolium tetrafluoroborate ($BMIMBF_4$), as suggested for published phenylglycine resolutions,[14,15] did not improve the enantiomeric purity (Table 1, entry 2). Among the other hydrolases, only chymotripsin afforded a promising result, with acid (*S*)-**2** being isolated in 40% conversion with 67% ee, that could not be improved, when modifying the reaction conditions (Table 1, entries 7–9). A Korean group has recently patented an alcalase[†]-catalyzed resolution of 2-chlorophenylglycine methyl ester,[18] but the subtilisin proteases (from *Bacillus licheniformis* or Carlsberg from *Bacillus subtilis*, Scheme 1), in our hands, afforded only partial enrichment in (*S*)-**3** (23–27% ee). It is worth noting that in this case the prevalent (*S*)-isomer is the unreacted ester. (see Table 1, entries 10, 11). This fact could be explained by the reported observation that subtilisin-proteases have the catalytic site arranged as the mirror image of lipase's catalytic site.[19]

CAL A and B were able to acylate (*RS*)-2-chlorophenylglycine methylester **3** to **4a** or **4b** in organic solvents, but, as reported for phenylglycine methylester with PPL and PFL,[20] their action was not selective. Acylases from *Aspergillus melleus*, *Streptomyces toyokaensis*, and porcine kidney did not recognize the *N*-acetyl derivative **4c** as the substrate and transformed *N*-trifluoroacetyl **4d** into **2** in low yield (10% after 24 h), without stereoselectivity.

These preliminary results prompted us to address our screening to an N-protected methylester, choosing the easily removable (by TFA treatment) *tert*-butoxycarbonyl group (Boc). From the lipases tested, only PPL showed a very low activity, transforming *N*-Boc methylester **6** into carboxylic acid **5** in low yield (10% after 10 days, 54% ee) (Table 2, entry 1). Chymotripsin, which was a more promising enzyme in the case of unprotected methylester **3**, did not transform the *N*-Boc derivative **6**, even after four days.

The best results were observed when the hydrolysis was performed, independently, by means of proteases either from *B. licheniformis* or from *B. Carlsberg*. In both cases, when 40% conversion was achieved (65 and 63 h) enantiomerically pure acid **5** was

[†] Alcalase is the crude subtilisin from NOVO containing mainly subtilisin Carlsberg.




**Scheme 2.** Reagents and conditions: (i) $CH_3OH$, DCC, DMAP, $CH_2Cl_2$; (ii) TFA, $CH_2Cl_2$; (iii) 20% $NH_4OH$; (iv) TsCl, $(iPr)_2O$, $Et_3N$; (v) $NaHCO_3$, KI, $CH_3CN$; (vi) paraformaldehyde, $ClCH_2CH_2Cl$, HCl in DMF.

**Table 3**
HPLC conditions

| Compound[a] | Column | Mobile phase[b] | Sample[c] | $R_T$ (min) | |
|---|---|---|---|---|---|
| | | | | (*R*)-Isomer | (*S*)-Isomer |
| **2** | Daicel Crownpack CR(+) 0.4 cm × 15 cm | $10^{-2}$ M $HClO_4$ | 0.1 mg/mL 0.2% HCOOH in $H_2O$ | 2.71 | 5.02 |
| **3a** | | | | 12.49 | 21.35 |
| **5** | Waters Spherisorb 5 μm OD 0.46 cm × 25 cm | $H_2O/CH_3CN/HClO_4$ 1:1:0.1 | 0.15 mg/mL $CH_3OH$ | 10.90 | |
| **6** | | | | 5.71 | |
| **9** | Daicel Chiralpak IA 0.46 cm × 25 cm | Hexane/EtOAc/$Et_2N$ 95:5:0.1 | 0.3 mg/mL EtOAc | 17.28 | 15.00 |
| **1** | Agilent Lichrocart 250-4 Chiradex 5 μ | $CH_3CN/CH_3OH$/0.01 M $KH_2PO_4$ 15:5:80 | 0.75 mg/mL mobile phase | 32.42 | 26.40 |

[a] Detector wavelength was set at 220 nm for all compounds, with exception of **9** (250 nm).
[b] Flow rate was of 1 ml/min, with exception of **9** (0.7 ml/min).
[c] 10 μl of each sample was injected.

isolated (Table 2, entries 2 and 3). In this case the (*S*)-configuration belongs to the acid, whereas in the case of unprotected ester **3** was the (*R*)-isomer which was transformed. The (*S*)-configuration of **5** was established after esterification to (*S*)-**6**, by comparison of its $[\alpha]_D$ value with the reported data.[21]

These satisfactory results were in agreement with the reported ones, by Arosio et al., who reported a detailed study of the protease-catalyzed hydrolysis of a series of arylglycines thioesters.[22] The ethylthioester, instead of a carboxyester, was chosen by the authors since it was suitable for complete substrate deracemization through an in situ base-catalyzed continued racemization of the (*R*)-isomer. The use of bad smelling ethanthiol was judged unsuitable for large-scale preparation purposes while on the other hand, considering the low cost of (*RS*)-2-chlorophenylglycin toward the final product value, the complete deracemization process was not mandatory; in any case the (*R*)-isomer could be later racemized and recycled. Since for the clopidogrel synthesis we needed the (*S*)-methyl ester and not the (*S*)-acid we tried to use subtilisin Carlsberg as the biocatalyst in the opposite reaction, that is, the esterification of acid **5**: in fact, in this case, due to the enzyme (*S*)-enantiopreference, the (*S*)-ester **6** should be obtained. Several examples of subtilisin-catalyzed esterification of aminoacids[23] or peptides[24] have been reported but in our case only the starting material was recovered in all the conditions tested. Since these results could be due to the negative effects of the methanol on the enzyme[25] we attempted to use an already known, more stable preparation of subtilisin, the cross-linked enzyme aggregation (CLEA) commercialized (Novozymes) with the Alcalase-CLEA® name;[26–28] however in this case no esterification of **5** to **6** was observed. However, the experimental advantages offered by the CLEA-subtilisin (e.g., the insoluble aggregate can be removed by simple paper filtration, thus avoiding tedious work-up of the emulsions obtained, when the free enzyme is used) prompted us to test this enzymatic preparation under hydrolytic conditions. From the examined organic solvents tetrahydrofuran afforded best results (>98% ee) while in acetonitrile and in dimethylformamide only 79% and 65% ee, respectively, were observed (Table 2, entries 4–6). The use of a cross-linked enzyme in THF/$H_2O$ also shortened the reaction time, the desired 40% conversion to (*S*)-acid **5** being achieved after 14 h, instead of the 63 h required in the case of free subtilisin in TBME/$H_2O$. The (*S*)-**5** isomer was converted, by esterification (methanol, DCC, DMAP in dichloromethane), and removal of the protecting group (TFA) into (*S*)-methyl ester **3** (90% yield), the synthon for the preparation of clopidogrel **1**.

## 2.2. Synthesis of clopidogrel 1 from (*S*)-3

From the reported syntheses of clopidogrel that provide the tetrahydrothienopyridine moiety formation as a first step, followed by the introduction of a suitable phenylacetic derivative[29] or, alternatively, delay the tetrahydropyridine ring formation after the reaction between a derivative of thiophenethanol **7** and (*S*)-[8] or (*RS*)-[9]methyl ester **3**, we chose the second approach, having already prepared enantiomerically pure **3**. Chemo-enzymatically prepared (*S*)-**3** was treated, following the literature method described for (*RS*)-**3**,[9] with tosylate **8** in acetonitrile in the presence of sodium hydrogen carbonate to afford intermediate **9** (70%). The best results (50% yield) for the heterocyclic ring formation were obtained with paraformaldehyde[29] (as the formaldehyde source) formalin[9] or 1,3-dioxolane affording lower yields and a more complex final reaction mixture (Scheme 2). The ee of (*S*)-**1** was determined by HPLC analysis on a chiral column.

### 2.3. HPLC analyses

In the case of N-unprotected 2-chlorophenylglycine methylester **3**, the enzymatic reaction progress could be monitored, contemporary to the ee evaluation, using a chiral column.

In the case of *N*-Boc derivatives, a chiral column screening had some difficulties (very long retention times and broad peaks) in performing the two evaluations at the same time; we thus decided to control the reaction progress by means of a C18 column, delaying the ee evaluation until after the Boc group removal. In this way we were able to not only detect the stereochemical outcome of the biotransformation but also verify if some racemization eventually occurred during the deprotection step.

The known arylglycines tendency for racemization suggested that we check the ee of not only the final product **1**, but also of all the intermediates. In fact, when we tried to prepare (*S*)-**3** as a free base (necessary for the nucleophilic substitution on tosylate **8**) from the trifluoroacetic salt obtained from Boc removal, the use of 0.5 M sodium hydrogen carbonate caused the formation of the (*R*)-isomer (10%). On the contrary, upon treatment with 20% ammonium hydroxide, no racemization was observed. By using chiral stationary phases, optical purity of intermediate **9** and final product **1** was also determined. Chosen columns, mobile phases, and retention times are collected in Table 3.

## 3. Conclusion

Enantiomerically pure clopidogrel **1**, an antiaggregatory drug structurally related to the nonproteinogenic aminoacid (*S*)-2-chlorophenylglycine **2**, was prepared in 32% yield starting from (*S*)-2-chlorophenylglycine methyl ester **3**. This chiral synthon was in turn obtained via a chemo-enzymatic approach, in order to avoid the frequent racemization process characteristic of arylglycines, due to the presence of a benzylic proton at the α-position. The best results were observed by means of a stereoselective protease-catalyzed hydrolysis of the easily available (*RS*)-**6**. The use of a cross-linked aggregate of subtilisin (Alcalase-CLEA®), instead of a free enzyme, not only simplified the experimental procedure and the enzyme recovery but also appreciably shortened the reaction time (14 h instead of 63 h), with comparable enantiomeric excesses. In order to evaluate the enantiomeric purities of all the intermediates, an HPLC method on the more convenient chiral stationary phases was studied for each compound. The good yields of the process, and the commercial availability of (*RS*)-2-chlorophenylglycine and CLEA-subtilisin, together with the efficiency of the enzymatic step make this synthetic pathway suitable for large-scale preparations, avoiding the tedious and time-consuming fractional crystallization of diastereomeric salts procedures, normally used for the (*S*)-isomer resolution.

## 4. Experimental

### 4.1. General

All the reagents and enzymes were purchased from Sigma–Aldrich. All reactions were monitored by TLC on Silica Gel 60 $F_{254}$ precoated plates with a fluorescent indicator (Merck) with detection (unless otherwise indicated) with a ninhydrin solution (0.3 g in butanol, 100 mL, and acetic acid, 3 mL) and heating at 110 °C. The TLC eluant was prepared by mixing water, butanol, and acetic acid (5:4:1), and separating the organic phase, after vigorous stirring. Column chromatography was performed on Silica Gel 60 (0.063–0.200 mm) (Merck). $^1$H NMR spectra were recorded on a Bruker-Avance 500 MHz spectrometer. Optical rotations were determined on a Perkin–Elmer 241 polarimeter in a 1 dm cell at 25 °C. HPLC analyses were performed with a Merck-Hitachi L-6200. Mass spectra were recorded on a Thermo Quest Finnigan LCQTM DECA ion trap mass spectrometer. Differential scanning calorimetry (DSC) was performed on a Perkin–Elmer DSC-7 instrument.

### 4.2. *N*-Boc-(*RS*)-2-chlorophenylglycine 5

To a suspension of 2-chlorophenylglycine **2** (10 g, 0.054 mol) in water (50 mL) 1,4-dioxane (40 mL) and sodium hydroxide (2.37 g, 0.059 mol) were added. After the addition of di-*tert*-butyl dicarbonate (12.4 mL, 0.054 mol), the mixture was kept at room temperature under stirring (18 h). The reaction progress was monitored in TLC. The mixture was concentrated at reduced pressure and 1 M hydrochloric acid was added until pH 3. The precipitated product (13.7 g, 89% yield) was recovered by suction. $^1$H NMR ($CDCl_3$) $\delta$ 1.23 (s, 9H, $CH_3C$), 5.75 (d, 1H, CH, *J* 4.5 Hz), 7.22–7.34 (m, 2H, Ar), 7.41 (d, 1H, Ar, *J* 6.5 Hz), 7.51 (d, 1H, Ar, *J* 6.9 Hz), 8.23 (d, 1H, NH, *J* 4.5 Hz).

### 4.3. *N*-Boc-(*RS*)-2-chlorophenylglycine methylester 6

To a solution of compound **5** (3 g, 10.5 mmol) in dry dichloromethane (80 mL), anhydrous methanol (0.85 mL), DCC (2.38 g, 11.54 mmol) and DMAP (0.12 g, 1.05 mmol) were sequentially added. The reaction mixture was kept under stirring at room temperature (2 h). The ester formation was monitored by TLC. The white precipitate was removed by suction. The filtrate was washed with a sodium hydrogen carbonate solution at pH 8 (2 × 20 mL), dried by treatment with sodium sulfate and evaporated at reduced pressure. The residue was purified by silica gel column chromatography (1/10) to afford pure **6** (eluant hexane/ethyl acetate 98:2, 2.7 g, 86%). $^1$H NMR ($CD_3OD$) $\delta$ 1.47 (s, 9H, $CH_3C$), 3.74 (s, 3H, $CH_3O$), 5.73 (br s, 1H, CH), 7.30–7.38 (m, 2H, Ar), 7.43 (m, 1H, Ar), 7.50 (m, 1H, Ar).

### 4.4. *N*-Boc-(*S*)-2-chlorophenylglycine 5

#### 4.4.1. By subtilisin-catalyzed hydrolysis of (*RS*)-6

To a solution of (*RS*)-**6** (1 g, 3.34 mmol) in TBME (18 mL), pH 7.5 buffer (0.1 M $KH_2PO_4$/0.1 M NaOH 50 mL/40.9 mL) (36 mL) and protease from *B. licheniformis* (24 mg, 255 U) were added. The reaction was kept under stirring, at 35 °C, and pH was maintained at 7.5 by the addition of 0.5 M sodium hydroxide. The reaction progress was monitored by HPLC (see Table 3). The samples for HPLC analysis were prepared as follows: an amount of aqueous and organic phase, 20 μl and 10 μl, respectively, was withdrawn and after collection, TBME was removed by means of a nitrogen stream. After the addition of methanol, the mixture was filtered through a 0.45 μm GHP ACRODISC and the resultant solution was analyzed. At 40% conversion (65 h) the pH was brought at 8 and the aqueous phase, after separation from the organic one, was extracted with TBME (5 × 15 mL). Extraction with TBME of the aqueous phase at pH 3, drying with sodium sulfate, filtration and evaporation under reduced pressure afforded (*S*)-acid **5** (0.33 g, 35%). $[\alpha]_D$ = +101 (*c* 1, $CH_3OH$). MS (ESI-) *m*/*z* 284 (M−1 with $^{35}$Cl), 286 (M−1 with $^{37}$Cl).

A sample was treated with TFA, as described below for compound **6**, and analyzed by HPLC (Table 3) in order to determine the ee (>99%).

#### 4.4.2. By Alcalase-CLEA®-catalyzed hydrolysis of (*RS*)-6

To a solution of (*RS*)-**6** (1 g, 3.34 mmol) in tetrahydrofuran (9 mL), water (90 mL) was added and the pH was adjusted to 8 with 2 M sodium hydroxide. Alcalase-CLEA (10 g, 255 U) was added and the mixture was kept under stirring at 30 °C, while maintaining the pH at 8 by the addition of 2 M sodium hydroxide,

monitoring the reaction progress by HPLC (see Table 3). The samples for HPLC analyses were prepared by the extraction of an amount of reaction mixture, at pH 3, evaporation of the solvents and dilution of the residue with methanol. At 40% conversion (14 h), the reaction mixture was extracted with TBME (3 × 80 mL); the aqueous phase was filtered by suction to remove the enzyme and after acidification to pH 3, extracted with TBME (4 × 60 mL). The organic phase was treated with sodium sulfate and, after filtration, evaporated under reduced pressure, to afford (*S*)-acid **5** (0.32 g, 34%). A sample, after removal of the protecting group to give the salt of compound **2**, was analyzed by HPLC (see Table 3) showing 98% ee.

### 4.5. *N*-Boc-(*S*)-2-chlorophenylglycine methylester 6

Esterification of (*S*)-acid **5** (0.6 g, 2.10 mmol) was performed following the procedure previously described for (*RS*)-**5**. The crude (*S*)-methylester **6** (0.6 g, 95%) was used in the next step without any further purification. A sample was purified for analytical purposes. $[\alpha]_D$ = +119.3 (*c* 1, $CHCl_3$) lit.[21] $[\alpha]_D$ = +117.1.

### 4.6. (S)-2-Chlorophenylglycine methylester 3

To a solution of (*S*)-**6** (0.5 g, 1.67 mmol) in dichloromethane (10 mL), under stirring at room temperature, a solution of TFA (0.64 mL, 8.35 mmol) in dichloromethane (10 mL) was added dropwise. The reaction progress was monitored by TLC until the disappearance of the starting material (3 h). The solvent was removed at reduced pressure. To the residue (98% ee by HPLC) dissolved in water (10 mL), 20% ammonium hydroxide was added until pH 7. Extraction with dichloromethane (3 × 20 mL), followed by treatment with sodium sulfate, filtration and evaporation of the solvent afforded the title compound **3** [0.315 g, 90% from (S)-**5**, 98% ee]. $[\alpha]_D$ = +123 (*c* 1, $CH_3OH$) lit.[30] $[\alpha]_D$ = +134. $^1$H NMR ($CDCl_3$) $\delta$ 2.3 (m, 2H, $NH_2$), 3.75 (s, 3H, $CH_3O$), 5.06 (br s, 1H, CH), 7.25–7.33 (m, 2H, Ar), 7.36–7.43 (m, 2H, Ar).

### 4.7. 2-(2-Thienyl)ethyl 1-*p*-tolylsulfonate 8

*p*-Toluenesulfonyl chloride (4.55 g, 23.87 mmol) and triethylamine (3.36 mL, 24.1 mmol) were added to a solution of 2-(2-thienyl)-ethanol **7** (3 g, 23.4 mmol) in diisopropylether (23 mL) and kept under stirring at room temperature until starting material disappearance (50 h, TLC toluene/ethyl acetate 9:1, detection with a 5% phosphomolybdic acid ethanol solution). The organic phase was washed with water (15 mL), 30% potassium carbonate aqueous solution (10 mL) and water (2 × 10 mL) and, then, dried over sodium sulfate, filtered, and evaporated under reduced pressure to afford tosylate **8** (5.85 g, 86%). $^1$H NMR ($CDCl_3$) $\delta$ 2.47 (s, 3H, $CH_3$Ar), 3.20 (t, 2H, $CH_2CH_2O$, *J* 7 Hz), 4.25, (t, 2H, $CH_2O$, *J* 7 Hz), 6.82 (d, 1H, H-3′, *J* 3.4 Hz), 6.92 (m, 1H, H-4′), 7.16 (d, 1H, H-5′, *J* 5 Hz), 7.34 (d, 2H, Ar, *J* 8 Hz), 7.78 (d, 2H, Ar, *J* 8 Hz).

### 4.8. Methyl (*S*)-2-(2-chlorophenyl)-2-[2-(thien-2-yl)ethylamino]-acetate 9

To a solution of **3** (2.2 g, 11.0 mmol) in acetonitrile (11 mL) tosylate **8** (2.8 g, 9.9 mmol), sodium hydrogen carbonate (1.1 g, 13.2 mmol) and potassium iodide (0.18 g, 1.1 mmol) were added. The mixture was kept under stirring at reflux (14 h). After this time an additional amount of potassium iodide (0.18 g) was added and the reaction was continued for another 6 h at reflux, while monitoring the reaction progress by TLC (toluene/ethyl acetate 95:5, detection with 5% phosphomolybdic acid ethanol solution). The solvent was evaporated at reduced pressure and the residue was dissolved with ethyl acetate (30 mL); the organic phase was washed with water (10 mL) and 15% sodium chloride aqueous solution (15 mL). After drying over sodium sulfate and filtration, the solvent was removed under reduced pressure. Crude residue **9** was purified by silica gel column chromatography (1/10). Elution with hexane/ethyl acetate 9:1 afforded pure **9** (2.38 g, 70%). Ee 98% (by HPLC, see Table 3). $^1$H NMR ($CDCl_3$) $\delta$ 2.21 (m, 1H, NH), 2.82 (m, 1H, CH–N), 2.96 (m, 1H, CH–N), 3.07 (t, 2H, $CH_2$-thienyl, *J* 7 Hz), 3.72 (s, 3H, $CH_3O$), 4.98 (br s, 1H, *CH*–$COOCH_3$), 6.85 (d, 1H, H-3′, *J* 3.4 Hz), 6.94 (m, 1H, H-4′), 7.15 (d, 1H, H-5′, *J* 5 Hz), 7.23–7.32 (m, 2H, Ar), 7.35–7.43 (m, 2H, Ar). MS (ESI+) *m*/*z* 310 (M+1 with $^{35}$Cl), 312 (M+1 with $^{37}$Cl).

### 4.9. Clopidogrel 1

To a solution of **9** (1 g, 3.23 mmol) in 1,2-dichloroethane (20 mL) paraformaldehyde (0.11 g, 3.67 mmol) was added; the mixture was kept under stirring, at reflux, removing water under azeotropic conditions for 4 h. After cooling to 30 °C, a solution (10 mL) of hydrochloric acid in dimethylformamide (4 g of HCl in 100 mL of DMF) was added dropwise. The mixture was heated at reflux (2 h) while monitoring the reaction progress by TLC (toluene/ethyl acetate 95:5, detection with 5% phosphomolybdic acid ethanol solution). After cooling at room temperature, water (200 mL) was added and the pH was adjusted to 7.5 by means of 30% aqueous potassium carbonate solution. Extraction with ethyl acetate (3 × 100 mL), followed by washing with water (100 mL), drying over sodium sulfate, filtration and removal of the solvent at reduced pressure gave a residue (0.67 g) that was purified by silica gel column chromatography (1/10, hexane/ethyl acetate 98:2 as eluant) affording pure **1** (0.52 g, 50%) as an oil. $^1$H NMR ($CDCl_3$) $\delta$ 2.87–2.96 (m, 4H, H-6 and H-7), 3.68 (d, 1H, H-4, *J* 4 Hz), 3.75 (s, 3H, $CH_3O$), 3.80 (d, 1H, H-4, *J* 4 Hz), 4.97 (br s, 1H, H-8), 6.68 (d, 1H, H-3, *J* 5 Hz), 7.08 (d, 1H, H-2, *J* 5 Hz), 7.24–7.36 (m, 2H, H-12 and H-13), 7.44 (d, 1H, H-14, *J* 7.7 Hz), 7.74 (d, 1H, H-11, *J* 7.7 Hz). MS (ESI+) *m*/*z* 322 (M+1 with $^{35}$Cl), 324 (M+1 with $^{37}$Cl). $[\alpha]_D$ = +45 (*c* 1, $CH_3OH$). Ee 98% (by HPLC). For analytical purposes a sample was transformed into the corresponding hydrogen sulfate $[\alpha]_D$ = +54.8 (*c* 1, $CH_3OH$) lit.[31] $[\alpha]_D$ = +55. DSC (5 °C/min) endothermic peak of fusion at 178.50 °C ($\Delta H$ 75.769 J/g).

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