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Lipase-catalyzed dynamic kinetic resolution of dimethyl (1,3-dihydro-2H-isooindol-1-yl)phosphonate

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\[ \text{PO(O\text{Me})_2} \quad \text{Candida antarctica} \quad \text{lipase type A} \quad \text{Organic solvent} \quad 30 \, ^\circ\text{C}, 250 \, \text{rpm} \quad \text{up to 77\% conversion up to 96\% ee} \]
AN ARTICLE INFO

A simple dynamic kinetic resolution of dimethyl (1,3-dihydro-2H-isoindol-1-yl)phosphonate has been developed by means of a lipase-catalyzed alkoxy carbonylation reaction. The influence of reaction parameters such as solvent, type and amount of alkoxy carbonylating agent, source and loading of enzyme, substrate concentration, temperature, and reaction time has been studied. The best results were found in the biocatalyzed reaction using Candida antarctica lipase type A and allyl 3-methoxyphenyl carbonate in toluene at 30 ºC, yielding the (R)-allyl carbonate in 58% isolated yield and 96% enantiomeric excess. Remarkably, this procedure does not require external auxiliaries for the racemization of the slow-reacting aminophosphonate enantiomer and occurs under mild reaction conditions.

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1. Introduction

The design of dynamic kinetic resolutions (DKRs) is an attractive manner to afford enantiopure compounds in theoretically 100% yield starting from racemates. These processes usually require the proper combination of a desired transformation with the in situ racemization of the slow-reacting enantiomer, so the action of mainly chiral auxiliaries, organocatalysts and metal-catalysis has been widely explored in recent decades. Biocataytic transformations have gained increasing attention in the development of sustainable DKR processes due to the compatibility of chemical and enzymatic catalysts for selected reactions, enabling the asymmetric transformation of racemic alcohols, amines and carboxylic acid derivatives using mainly hydrolyses as biocatalysts. More recently, the use of other classes of enzymes such as alcohol dehydrogenases and transaminases have opened a myriad of opportunities in the development of efficient DKR processes.

The design of enzyme-catalyzed DKRs under spontaneous conditions is especially attractive as simplifies the overall process without requiring the addition of external catalyst (i.e. bases, metal-complexes, auxiliaries, etc.). This fact facilitates not only the optimization of the enzymatic process but also the isolation of the aimed optically active compound, avoiding undesired epimerization processes in some cases. Without any doubt, the presence of labile hydrogen atoms in the structural core of the racemate determines the possible application of this methodology. For instance, our group has recently reported the DKR of 1,3-dihydro-2H-isoidol-1-carboxylic acid methyl ester taking advantage of a deprotonation-protonation process of the achiral intermediate, which occurs under Pseudomonas cepacia lipase-catalyzed alkoxy carbonylation conditions (Figure 1 left). These analogues of α-amino acids possess a phosphonic acid group rather than the carboxylic acid functionality. As a result, α-amino phosphonic acids and peptides incorporating them exhibit a broad range of biological and pharmacological activities. The outstanding properties of α-amino phosphonic acids and phosphonates have stimulated the development of synthetic strategies towards the preparation of these compounds, being of special interest those leading to optically active compounds.

Herein we wish to explore the enzyme-catalyzed resolution of the phosphonic counterpart of 1,3-dihydro-2H-isoidol-1-carboxylic acid methyl ester, this is dimethyl (1,3-dihydro-2H-isoidol-1-yl)phosphonate (Figure 1 middle), a phosphoproline analogue which is present in the structure of selective irreversible inhibitors of dipeptidyl peptidases 8 (DPP8) and 9 (DPP9). To the best of our knowledge, the biocatalyzed resolution of cyclic α-amino phosphonic acid derivatives has been only reported in the lipase-catalyzed alkoxy carbonylation of dimethyl pyrrolidine-2-phosphonate (Figure 1 right), finding moderate selectivity values after an exhaustive enzymatic study.
2. Results and discussion

The synthesis of racemic dimethyl (1,3-dihydro-2H-isodindol-1-yl)phosphonate (2) was performed starting from commercially available phthalimide. This procedure involves a three-step sequence towards the N-Boc protected precursor 1,14 which was deprotected under acidic conditions in 90% yield (Scheme 1). Prior to develop enzymatic transformations, the allyl and benzyl racemic carbamates 4a-b were prepared using allyl chloroformate (3a) or benzyl chloroformate (3b) in 70% and 99% isolated yield respectively. These racemates were only used for analytical purposes in the development of reliable chiral methods for the measurement of the enantiomeric excess values once the biocatalyzed products were obtained.

![Scheme 1. Chemical synthesis of racemic dimethyl (1,3-dihydro-2H-isodindol-1-yl)phosphonate and related allyl and benzyl carbamates 4a,b.](image)

Based on the excellent results found in the resolution of 1,3-dihydro-2H-isodindole-1-carboxylic acid methyl ester,8 the resolution of aminophosphonate 2 was attempted through an alkoxycarbonylation reaction using a series of lipases that includes *Pseudomonas cepacia* lipase (PSL-C 1), *Candida antarctica* lipase type B (CAL-B) and *Candida antarctica* lipase type A (CAL-A). The enzymatic screening was conducted at a 140 mM substrate concentration in tert-butyl methyl ether (MTBE) at 30 °C (Table 1), initially using commercially available diallyl carbonate (5a). In contrast with the excellent activity displayed towards the carbamoylation of 1,3-dihydro-2H-isodindole-1-carboxylic acid methyl ester,8 PSL-C 1 did not display any activity against the homologue phosphonate 2 (entry 1). Similarly, the racemic starting material was recovered untouched in the reaction with CAL-B (entry 2). Satisfyingly, CAL-A catalyzed the formation of (R)-4a in 78% ee and 50% conversion after 92 h (entry 3), obtaining the remaining aminophosphonate (S)-2 in 26% ee. This poor optical purity of the substrate at a considerable conversion value suggests its racemization in the reaction medium, so the possibility of achieving a suitable DKR process was opened at this time. We propose a deprotonation-protonation equilibrium through an achiral enolate intermediate, as occurs with its 1,3-dihydro-2H-isodindole-1-carboxylic acid methyl ester analogue.8

Then, the enzymatic study was continued employing a series of carbamates, such as commercially available dibenzyl carbonate (5b) and easily to prepare allyl 3-methoxyphenyl carbonate (5c)15 in combination with CAL-A. On the other hand, the dibenzyl carbonate was used although no conversion was observed (entry 4). On the other hand, high conversions and selectivities were observed with the more reactive allyl 3-methoxyphenyl carbonate 5c (entries 5 and 6), obtaining the (R)-allyl carbamate 4a in 58% isolated yield and 96% ee after column chromatography, while the substrate was recovered almost in racemic form when the reaction was carried out in toluene as solvent. It must be noticed that the optical purity of the amino phosphonate decreased at prolonged times under air conditions, demonstrating the lability of the hydrogen atom in the α-position to the phosphonate group. In all cases, the remaining amino phosphonate 2 and the corresponding carbamate were found as unique products in the biotransformation, although a side hydrolysis reaction or decomposition are not discarded during the purification step using a column chromatography on silica gel.

### Table 1. Asymmetric alkoxycarbonylation of racemic aminophosphonate 2 using different lipases (ratio 2:1 lipase:substrate w/w) and 2.5 equiv. of alkyl carbonates at 30 °C and 250 rpm.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Lipase</th>
<th>Solvent</th>
<th>Carbamate 5a-c</th>
<th>t (h)</th>
<th>c (%)a</th>
<th>ee (%)a</th>
<th>ee (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PSL-C 1</td>
<td>MTBE</td>
<td>5a</td>
<td>24</td>
<td>&lt;1</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>CAL-B</td>
<td>MTBE</td>
<td>5a</td>
<td>48</td>
<td>&lt;1</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>3</td>
<td>CAL-A</td>
<td>MTBE</td>
<td>5a</td>
<td>92</td>
<td>50</td>
<td>78</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>CAL-A</td>
<td>MTBE</td>
<td>5b</td>
<td>24</td>
<td>&lt;1</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>5</td>
<td>CAL-A</td>
<td>MTBE</td>
<td>5c</td>
<td>27</td>
<td>67</td>
<td>87</td>
<td>32</td>
</tr>
<tr>
<td>6</td>
<td>CAL-A</td>
<td>Toluene</td>
<td>5c</td>
<td>47</td>
<td>77</td>
<td>96 (58)</td>
<td>9</td>
</tr>
</tbody>
</table>

a Conversion values determined by 1H-NMR of the reaction crudes. Isolated yields in parentheses.

b Enantiomeric excess values determined by HPLC analysis.
Table 2. Dynamic kinetic resolution of racemic aminophosphonate 2 using CAL-A and allyl 3-methoxyphenyl carbonate in toluene at 250 rpm.

<table>
<thead>
<tr>
<th>Entry</th>
<th>CAL-A (w/w)</th>
<th>5c (equiv.)</th>
<th>[(±)-2] (mM)</th>
<th>T (°C)</th>
<th>t (h)</th>
<th>c (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2:1</td>
<td>2.5</td>
<td>140</td>
<td>30</td>
<td>47</td>
<td>77</td>
<td>96</td>
</tr>
<tr>
<td>2</td>
<td>4:1</td>
<td>2.5</td>
<td>140</td>
<td>30</td>
<td>46</td>
<td>62</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>2:1</td>
<td>2.5</td>
<td>140</td>
<td>45</td>
<td>47</td>
<td>68</td>
<td>94</td>
</tr>
<tr>
<td>4</td>
<td>2:1</td>
<td>2.5</td>
<td>280</td>
<td>30</td>
<td>46</td>
<td>59</td>
<td>91</td>
</tr>
<tr>
<td>5</td>
<td>2:1</td>
<td>10</td>
<td>140</td>
<td>30</td>
<td>71</td>
<td>57</td>
<td>85</td>
</tr>
</tbody>
</table>

a Ratio CAL-A:aminophosphonate (w/w).
b Conversion values determined by 1H-NMR of the reaction crudes.
c Enantiomeric excess values determined by HPLC analysis.

Exploring other reaction conditions for the asymmetric synthesis of the allyl carbamate (R)-4a, a series of reaction parameters were considered such as an increase in enzyme and carbonate 5c loadings, and the use of higher substrate concentrations and temperatures (Table 2). Doubling the amount of enzyme did not affect significantly the reaction, observing a slight reduction of the conversion but maintaining the selectivity (entries 1 and 2), while the use of higher temperatures did not significantly affect the final results (entry 3). A higher substrate concentration (280 mM) did not lead to a beneficial impact either in the kinetic or the selectivity of the process (entry 4). Finally, the DKR was attempted with a significant excess of the carbonate 5c but the enantiomeric excess of the carbamate and the conversion were considerably lower even at prolonged reaction times (entry 5).

Absolute configuration of the allyl carbamate 4a resulting from the resolution catalyzed by CAL-A at 30 ºC (entry 6, Table 1) was determined by 1H-NMR analyses. The synthesis of the corresponding Mosher amide derivative 6 was required, firstly starting from racemic 2 and later using the allyl carbamate 4a obtained in 96% ee under enzyme-catalyzed DKR conditions (Scheme 2). Derivatization of the free aminophosphonate (±)-2 was performed employing the (S)-(+)α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) in the presence of triethylamine. Firstly, the mixture of diastereoisomeric Mosher amides (RS,R)-6 was prepared (Scheme 2 top), clearly finding two doublet signals in the 1H-NMR spectrum at 5.87 ppm (J_H,N = 9.0 Hz) and 5.99 ppm (J_H,N = 8.9 Hz) corresponding to the CH in α position to the nitrogen atom (Figure 2). The doublet signal at higher fields corresponds to the (R,R)-6 due to the magnetic anisotropy caused by the phenyl ring, while at lower fields the (S,R)-6 diastereoisomer was found with the hydrogen atom and the phenyl ring at opposite sides.

Secondly, the allyloxycarbonyl group of 4a obtained in the lipase-catalyzed alkoxy carbonylation was deprotected by reaction with 1,3-dimethylbarbituric acid (DMBA), palladium (II) acetate and triphenylphosphine (Scheme 2 bottom). Thus, the corresponding free aminophosphonate 2 was isolated and immediately derivatized using (S)-MTPA-Cl in the presence of triethylamine to afford the desired optically active Mosher amide 6 in good yield.

Comparison of the 1H-NMR spectra of the diastereoisomeric mixture of Mosher amides and the corresponding to the Mosher derivative of the optically active product (Figure 3), allowed us to define (R)-configuration for the chiral center in the isoindoline core, and then a (R,R)-stereochemistry for the Mosher amide 6. Partial epimerization was observed after the deprotection-derivatization sequence (91% diastereomeric excess from a starting 96% enantiomeric excess), highlighting the lability of this type of compound, although the racemization occurs in a little extent in comparison with the homologue methyl ester.

Figure 2. 1H-NMR spectrum window with representative signals for the mixture of diastereoisomeric Mosher amides (RS,R)-6. For simplicity [P] represents the dimethyl phosphonate functionality.
Tetrahedron

Scheme 2. Synthesis of Mosher amide derivatives for the determination of the absolute configurations of allyl carbamate 4a obtained in the biocatalyzed DKR using CAL-A.

Figure 3. 1H-NMR spectrum window with representative signals for the Mosher amide derivative (R,R)-6 isolated in 91% de. For simplicity [P] represents the dimethyl phosphonate functionality.

3. Conclusion

Lipase-catalyzed transformations have been studied for the resolution of dimethyl (1,3-dihydro-2H-isindol-1-yl)phosphonate. The approach was based on the alkoxyacylation reaction of the free amino group, developing a dynamic kinetic resolution process motivated by the in situ racemization of the slower reacting enantiomer in the biocatalytic process in the absence of auxiliaries. The combination of Candida antarctica lipase type A and allyl 3-methoxyphenyl carbonate using toluene as solvent under mild reaction conditions led to the recovery of the (R)-allyl carbamate in 58% isolated yield and 96% enantiomeric excess. A series of reaction parameters were studied searching for a global knowledge of the biocatalytic transformation, although in all cases conversions were up to 77% generally obtaining high selectivities. 1H-NMR analyses served for the assignment of the absolute configuration of the resulting (R)-allyl carbamate, after its convenient transformation into the corresponding Mosher amide derivative.

4. Experimental

4.1. General

Pseudomonas cepacia lipase type I immobilized over a ceramic support (PSL-C I, Amano, 1019 U/g), was purchased from Sigma-Aldrich. Candida antarctica lipase type B (CAL-B, Novozyme 435, 7300 PLU/g) was a gift from Novozymes (Denmark). Candida antarctica lipase A (CAL-A, 2.6 U/mg solid) was purchased from Codexis.

All other reagents and solvents were used as received from commercial suppliers without further purification. Column chromatographies were performed using silica gel 60 (230-240 mesh) purchased from Merck. Melting points were measured in a Gallenkamp apparatus, and taken on samples in open capillary tubes. IR spectra were recorded on a Varian 1000 FT-IR spectrometer using NaCl plates or KBr pellets. 1H, 13C and DEPT NMR experiments were performed using AV-300 and DPX-300 Bruker spectrometers at room temperature (1H, 300.13 MHz and 13C, 75.5 MHz). The chemical shifts are given in delta (δ) values and the coupling constants (J) in Hertz (Hz). High-resolution mass spectra were recorded on a Bruker Microtof-Q spectrometer. Enantiomeric excess values were determined through HPLC analyses using an Agilent 1100 Series chromatogram with a 20% of 2-propanol/hexane as eluent with a 0.8 mL/min flow at 30 °C. A Chiralcel OJ-H (25 x 4.6 mm i.d.) column was used for the allyl carbamate 4a [8.0 minutes for the (R)-enantiomer and 9.7 minutes for the (S)-enantiomer] and for the benzyl carbamate 4b [14.7 and 23.8 minutes], while a Chiralpak AS (25 x 4.6 mm i.d.) column was used for the free aminophosphonate 2 [13.1 minutes for the (R)-enantiomer and 19.1 minutes for the (S)-enantiomer].

4.2. Synthesis of dimethyl (1,3-dihydro-2H-isindol-1-yl)phosphonate, 2

A suspension of N-Boc protected aminophosphonate 1 (200 mg, 0.61 mmol) in an aqueous HCl 3 M solution (10 mL) was stirred at room temperature for 6 h until complete solubility of the mixture. The solution was basified with an aqueous NaOH 4 M solution (10 mL) and extracted with CHCl3 (3 x 15 mL). The organic phases were combined, dried over Na2SO4, filtered and the solvent was evaporated under reduced pressure, yielding the racemic and free-protected aminophosphonate 2 as a colourless viscous liquid (90%), Rf (70% EtOAChexane): 0.24, IR (NaCl) ν 3417, 1594, 1230, 1034 cm⁻¹. 1H-NMR (CDCl3, 300.13 MHz) δ 3.58 (d, 3JHH= 10.3 Hz, 3H, OMe), 3.75 (d, 4JHH= 10.4 Hz, 3H, OMe), 4.23-4.44 (m, 2H, Hs), 4.78 (d, 2JHH = 7.7 Hz, 1H, Hs), 5.26 (bs, 1H, NH), 6.19-7.29 (m, 3H, Ar), 7.46 (dd, 3JHH = 5.2, 4JHH = 1.8 Hz, 1H, Ar) ppm. 13C-NMR (CDCl3, 75.5 MHz) δ 52.5 (d, 3JHH = 2.4 Hz, CHs, 2Cl), 53.2 (d, 3JHH = 7.3 Hz, CHs, OMe), 53.6 (d, 3JHH = 7.2 Hz, CHs, OMe), 60.6 (d, 3JHH = 159.3 Hz, CH, Cs), 122.6 (d, JHH = 2.2 Hz, CH, Ar), 123.9 (d, JHH = 3.3 Hz, CH, Ar), 127.1 (d, JPC = 2.9 Hz, CH, Ar), 128.0 (d, JPC = 3.0 Hz, CH, Ar), 136.3 (d, 3JPC = 3.9 Hz, C, Cx), 141.7 (d, 3JPC = 7.2 Hz, C,
Pyridine (23.5 μL, 0.29 mmol, 1.11 equiv.) and allyl chloroformate (3a, 30.8 μL, 0.29 mmol, 1.11 equiv.) were successively added to a solution of racemic 2,3-dihydro-2H-isoindol-1-yl)phosphonate 4a.

Pyridine (23.5 μL, 0.29 mmol, 1.11 equiv.) and benzyl chloroformate (3b, 41.4 μL, 0.29 mmol, 1.11 equiv.) were successively added to a solution of racemic 2,3-dihydro-2H-isoindol-1-yl)phosphonate 4b.

4. Synthesis of dimethyl [N-(allyloxy carbonyl)-1,3-dihydro-2H-isoindol-1-yl]phosphonate, 4a

Pyridine (23.5 μL, 0.29 mmol, 1.11 equiv.) and allyl chloroformate (3a, 30.8 μL, 0.29 mmol, 1.11 equiv.) were successively added to a solution of racemic 2,3-dihydro-2H-isoindol-1-yl)phosphonate 4a (60 mg, 0.26 mmol) in dry dichloromethane (1 mL) and under inert atmosphere. The solution was stirred at room temperature for 2 h, and after this time the solvent was evaporated under reduced pressure. The reaction crude was purified through column chromatography on silica gel (80% EtOAc/hexane as eluent), yielding the carbamate 4a as a white solid (70% isolated yield). Rf (80% EtOAc/hexane): 0.30; Mp: 74-76 °C. 1H-NMR (CDCl3, 300.13 MHz) δ (duplicated signals are observed for some protons and they have been shown in brackets; asterisks indicate those ones corresponding to the minor rotamer) [3.45* (d, JPH2 = 10.4 Hz) + 3.52 (d, JPH2 = 10.5 Hz, 3H, OMe)], [3.77* (d, JPH2 = 10.7 Hz) + 3.85 (d, JPH2 = 10.7 Hz, 3H, OMe)], 4.59-4.63 (m, 3H, H3 + H1), 5.24 (d, JNH = 10.4 Hz, 1H, H2), 5.36 (dd, Jdd = 10.4 Hz, JNH = 17.1 Hz, 1H, H3), [5.50* (d, JNH = 6.4 Hz) + 5.54 (d, JNH = 6.6 Hz, 1H, H1)], 5.97 (ddt, Jdd = 16.4, 10.6, 5.5 Hz, 1H, H2), 7.26-7.35 (m, 3H, Ar), 7.45-7.56 (m, 1H, Ar) ppm. 13C-NMR (CDCl3, 75.5 MHz) δ (duplicated signals are observed for most of the carbon atoms and they have been shown in brackets; asterisks indicate those ones corresponding to the minor rotamer; dooublet signals for the methoxy rotamers appears overlapped) [52.5 + 52.8* (CH2, C2)], 53.5-53.7 (m, 2CH2, OMe), 59.8 (d, JPC = 158.8 Hz, CH, C), [66.6 + 66.8* (CH2, C2)], [117.9 + 118.3* (CH3), C], [122.6 + 122.7* (CH, Ar)], 124.3 (CH, Ar), 127.7 (CH, Ar), 128.5 (CH, Ar), 132.7 (CH, C), 134.1 (C, Ar), 137.9 (C, Ar), 154.7 (C, C=O) ppm. HRMS (ESI+, m/z) caleld for C15H15N2O2P+ (M+Na)+: 334.0815, found: 334.0825.

4.4. Synthesis of dimethyl [N-(benzylcarbonyl)-1,3-dihydro-2H-isoindol-1-yl]phosphonate, 4b

An erlenmeyer flask containing the racemic aminophosphonate 2 (100 mg, 0.44 mmol), the allyl 3-methoxyphenyl carbonate (5c, 228 mg, 1.10 mmol, 2.5 equiv.) and CAL-A (200 mg, 2:1 in weight respect to the substrate) was stopped with a septum and an inert atmosphere was generated with the aid of a needle. Dry toluene (3.1 mL) was added and the reaction mixture was shaken at 30 °C and 250 ppm for 47 h. The enzyme was removed by filtration, washed with CH2Cl2 (3 x 5 mL), and the solvents removed under reduce pressure to reach a 77% conversion value (1H-NMR of the reaction crude). The so-obtained reaction crude was purified by column chromatography on silica gel to obtain the carbamate (R)-4a (58% isolated yield, 96% ee) using a 80% EtOAc/hexane eluent, and the remaining aminophosphonate (S)-2 (9% ee) when eluting with a 100% EtOAc eluent. [α]D20 = −58.3 (c 0.3, EtOH) for 96% ee.

4.5. General procedure for the synthesis of the Mosher derivative (R, R)-6

The (S)-Mosher’s acid chloride (10 μL, 0.055 mmol, 1.25 equiv.) and dry Et2N (8 μL, 0.055 mmol, 1.25 equiv.) was successively added to a solution of racemic aminophosphonate 2 (10 mg, 0.044 mmol) in dry CH2Cl2 (0.5 mL) under inert atmosphere. The mixture was stirred for 10 h at room temperature and then CH2Cl2 (10 mL) was added. The solution was washed with H2O (2 x 10 mL), dried with Na2SO4, filtered and concentrated under reduced pressure. The resulting residue was analyzed by 1H-NMR in order to identify characteristic signals for diastereoisomers (R, R)-6 and (S, R)-6.

4.7. General procedure for the deprotection of the allyloxy carbonyl group of (R)-dimethyl [N-(allyloxy carbonyl)-1,3-dihydro-2H-isoindol-1-yl]phosphonate, 4a

N,N-Dimethylbarbituric acid (27 mg, 0.17 mmol, 2.7 equiv.), Pd(OAc)2 (1.5 mg, 0.0064 mmol, 0.10 equiv.) and PPh3 (5.0 mg, 0.019 mmol, 0.30 equiv.) were successively added to a solution of optically active carbamate (R)-4a (20 mg, 0.064 mmol, 96% ee) in dry CH2Cl2 (0.8 mL) under inert atmosphere. The mixture was stirred at reflux temperature during 1 h, and then it was slowly cooled until room temperature. Additional CH2Cl2 (10 mL) was added to the solution, which was washed with water (2 x 10 mL). The organic layer was dried with Na2SO4, filtered and concentrated under reduced pressure, yielding a reaction crude that mainly contains the free aminophosphonate (R)-2. A further purification of this reaction crude was discarded to avoid a possible racemization of the product.

4.8. General procedure for the synthesis of the Mosher derivative (R, R)-6

To a solution of the so-obtained residue from the removal of the allyloxy carbonyl group reaction (0.064 mmol for a theoretically 100% yield) in dry CH2Cl2 (0.7 mL) and under inert atmosphere, the (S)-Mosher’s acid chloride (15 μL, 0.080 mmol, 1.25 equiv.) and dry Et2N (11 μL, 0.080 mmol, 1.25 equiv.) were successively added. The mixture was stirred for 10 h at room temperature and then CH2Cl2 (10 mL) was added. The solution was washed with H2O (2 x 10 mL), dried with Na2SO4, filtered
and concentrated under reduced pressure. The resulting residue was purified by column chromatography on silica gel (50% EtOAc/hexane), yielding the corresponding Mosher derivative (R,R)-6 with 91% de (76% isolated yield).

Acknowledgments

Financial support to this work was provided by Ministerio de Economía y Competitividad-FEDER (grants CTQ2013-44153-P, and CTQ2013-40855-R), Gobierno de Aragón–FSE (research group E40) and Gobierno del Principado de Asturias (FC-15-GRUPIN14-002). M.L.-I. thanks FICYT for a predoctoral fellowship. A.A. thanks the Spanish Consejo Superior de Investigaciones Científicas for a JAE predoctoral fellowship. The authors thank Novo Nordisk Co. for the generous gift of CAL-B (Novozyme 435).

References and notes