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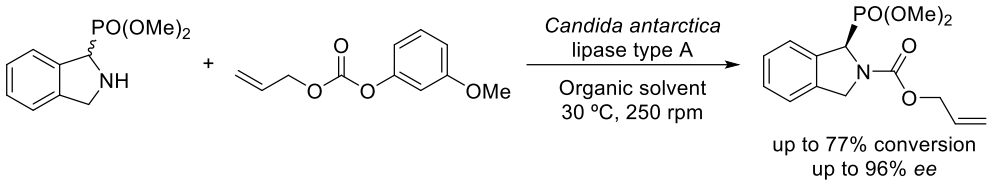

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Lipase-catalyzed dynamic kinetic resolution of dimethyl (1,3-dihydro-2*H*-isoindol-1-yl)phosphonate

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ABSTRACT

A simple dynamic kinetic resolution of dimethyl (1,3-dihydro-2*H*-isoindol-1-yl)phosphonate has been developed by means of a lipase-catalyzed alkoxycarbonylation reaction. The influence of reaction parameters such as solvent, type and amount of alkoxycarbonylating 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 carbamate 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.

1. Introduction

The design of dynamic kinetic resolutions (DKRs) is an attractive manner to afford enantiopure compounds in theoretically 100% yield starting from racemates.[1] 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.[2] Biocatalytic transformations have gained increasing attention in the development of sustainable DKR processes due to the compatibility of chemical and enzymatic catalysts for selected reactions,[3] enabling the asymmetrization of racemic alcohols, amines and carboxylic acid derivatives using mainly hydrolases as biocatalysts.[2,4] More recently, the use of other classes of enzymes such as alcohol dehydrogenases[5] and transaminases have opened a myriad of opportunities in the development of efficient DKR processes.[6]

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).[7] 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-2*H*-isoindole-1-carboxylic acid methyl ester taking advantage of a deprotonation-protonation process of the achiral intermediate, which occurs under *Pseudomonas cepacia* lipase-catalyzed alkoxycarbonylation conditions (Figure 1 left).[8]

Among biologically active compounds, α-aminophosphonic acids have attracted a special interest in the latest decades.[9] These analogues of α-amino acids possess a phosphonic acid group rather than the carboxylic acid functionality. As a result, α-aminophosphonic acids and peptides incorporating them exhibit a broad range of biological and pharmacological activities.[10] The outstanding properties of α-aminophosphonic acids and phosphonates have stimulated the development of synthetic strategies towards the preparation of these compounds,[11] 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-2*H*-isoindole-1-carboxylic acid methyl ester, this is dimethyl (1,3-dihydro-2*H*-isoindol-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).[12] To the best of our knowledge, the biocatalyzed resolution of cyclic α-aminophosphonic acid derivatives has been only reported in the lipase-catalyzed alkoxycarbonylation of dimethyl pyrrolidine-2-phosphonate (Figure 1 right), finding moderate selectivity values after an exhaustive enzymatic study.[13]

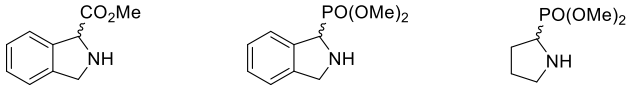
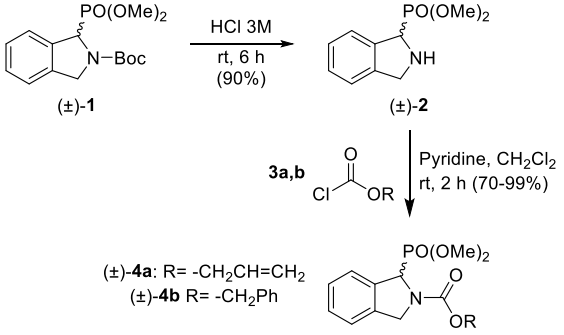


Figure 1. Structure of 1,3-dihydro-2*H*-isoindole-1-carboxylic acid methyl ester, dimethyl (1,3-dihydro-2*H*-isoindol-1-yl)phosphonate and dimethyl pyrrolidine-2-phosphonate.

2. Results and discussion

The synthesis of racemic dimethyl (1,3-dihydro-2*H*-isoindol-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.

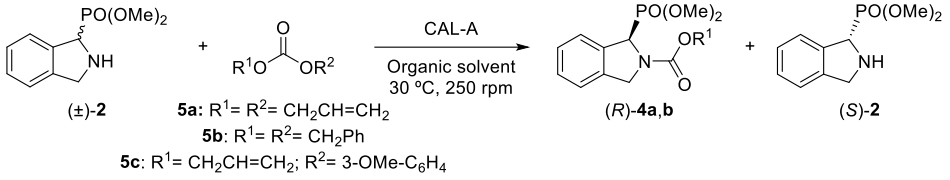


Scheme 1. Chemical synthesis of racemic dimethyl (1,3-dihydro-2*H*-isoindol-1-yl)phosphonate and related allyl and benzyl carbamates **4a**,**b**.

Based on the excellent results found in the resolution of 1,3-dihydro-2*H*-isoindole-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 I), *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 ºC (Table 1), initially using commercially available diallyl carbonate (**5a**). In contrast with the excellent activity displayed towards the carbamoylation of 1,3-dihydro-2*H*-isoindole-1-carboxylic acid methyl ester,[8] PSL-C I 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-2*H*-isoindole-1-carboxylic acid methyl ester analogue.[8]

Then, the enzymatic study was continued employing a series of carbonates, such as commercially available dibenzyl carbonate (**5b**) and easily to prepare allyl 3-methoxyphenyl carbonate (**5c**)[15] in combination with CAL-A. On the one 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.

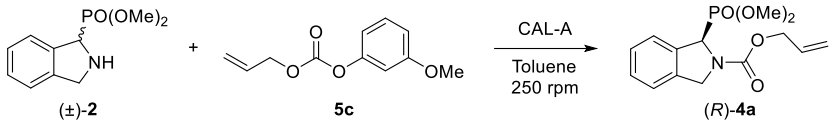
Table 1. Asymmetric alkoxycarbonylation of racemic aminophosphonate **2** using different lipases (ratio 2:1 lipase:substrate w/w) and 2.5 equiv. of alkyl carbonates at 30 ºC and 250 rpm.



Entry	Lipase	Solvent	Carbonate **5a-c**	*t* (h)	*c* (%)[a]	ee_P (%)[b]	ee_S (%)[b]
1	PSL-C I	MTBE	**5a**	24	<1	----	----
2	CAL-B	MTBE	**5a**	48	<1	----	----
3	CAL-A	MTBE	**5a**	92	50	78	26
4	CAL-A	MTBE	**5b**	24	<1	----	----
5	CAL-A	MTBE	**5c**	27	67	87	32
6	CAL-A	Toluene	**5c**	47	77	96 (58)	9

[a] Conversion values determined by ^{1}H-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.



Entry	CAL-A[a]	**5c** (equiv.)	[(±)-**2**] (mM)	T (ºC)	*t* (h)	*c* (%)[b]	ee_P (%)[c]
1	2:1	2.5	140	30	47	77	96
2	4:1	2.5	140	30	46	62	95
3	2:1	2.5	140	45	47	68	94
4	2:1	2.5	280	30	46	59	91
5	2:1	10	140	30	71	57	85

[a] Ratio CAL-A:aminophosphonate (w/w).
[b] Conversion values determined by ^{1}H-NMR of the reaction crudes.
[b] 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 ^{1}H-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 doblet signals in the ^{1}H-NMR spectrum at 5.87 ppm ($^2J_{PH}$ = 9.0 Hz) and 5.99 ppm ($^2J_{PH}$ = 8.9 Hz) corresponding to the CH in α position to the nitrogen atom (Figure 2). The doblet 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 alkoxycarbonylation 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 ^{1}H-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.[8]

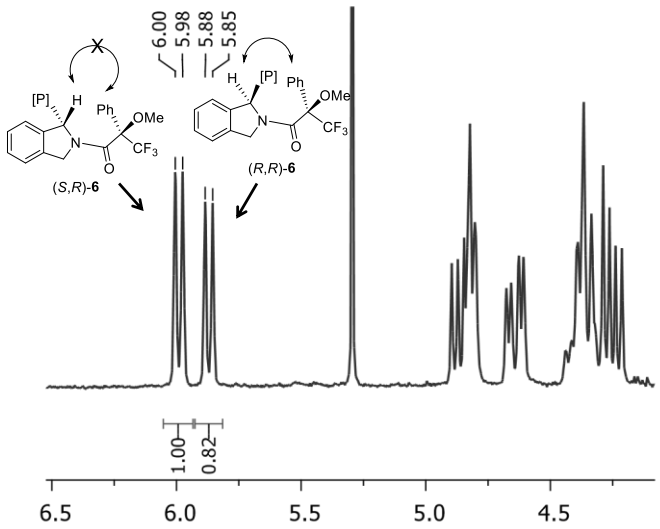
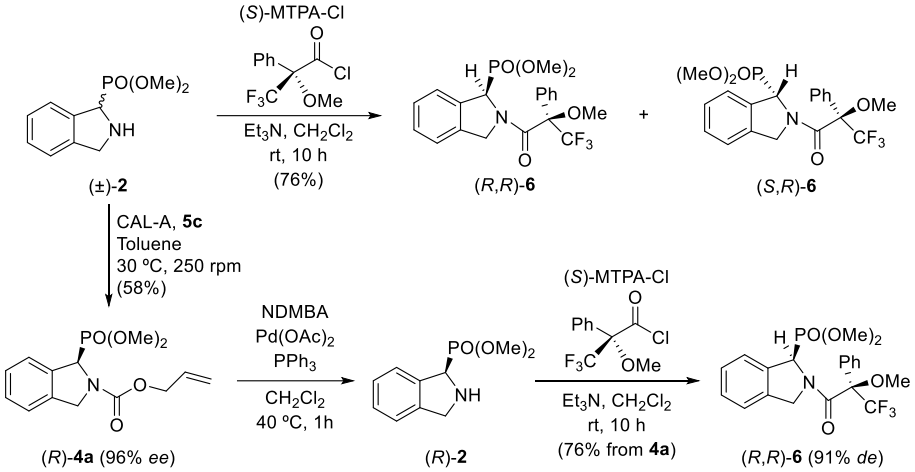


Figure 2. ^{1}H-NMR spectrum window with representative signals for the mixture of diastereoisomeric Mosher amides (*RS*,*R*)-**6**. For simplicity [P] represents the dimethyl phosphonate functionality.



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.

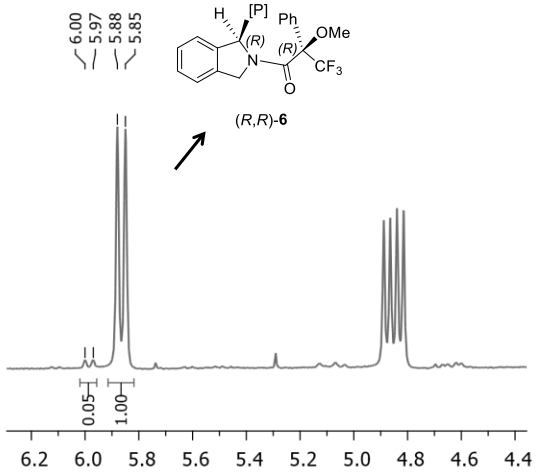


Figure 3. ^{1}H-NMR spectrum window with representative signals for the Mosher amide derivative (*R*,*R*)-**6** isolated in 91% *de*. For simplicity [P] represents the dimethyl phosphonate functionality.

3. Conclusion

Lipase-catalyzed transformations have been studied for the resolution of dimethyl (1,3-dihydro-2*H*-isoindol-1-yl)phosphonate. The approach was based on the alkoxycarbonylation 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 biocatalyzed transformation, although in all cases conversions were up to 77% generally obtaining high selectivities. ^{1}H-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. ^{1}H, ^{13}C and DEPT NMR experiments were performed using AV-300 and DPX-300 Brüker spectrometers at room temperature (^{1}H, 300.13 MHz and ^{13}C, 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 ºC. 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-2*H*-isoindol-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 CH_2Cl_2 (3 x 15 mL). The organic phases were combined, dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure, yielding the racemic and free-protected aminophosphonate **2** as a colourless viscous liquid (90%). R_f (70% EtOAc/hexane): 0.24. IR (NaCl) ν 3417, 1594, 1230, 1034 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz) δ 3.58 (d, $^{3}J_{PH}$= 10.3 Hz, 3H, OMe), 3.75 (d, $^{3}J_{PH}$= 10.4 Hz, 3H, OMe), 4.23-4.44 (m, 2H, H_3), 4.78 (d, $^{2}J_{PH}$ = 7.7 Hz, 1H, H_1), 5.26 (brs, 1H, NH), 7.19-7.29 (m, 3H, Ar), 7.46 (dd, $^{3}J_{HH}$ = 5.2, $^{4}J_{HH}$ = 1.8 Hz, 1H, Ar) ppm. ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ 52.5 (d, $^{3}J_{PC}$ = 2.4 Hz, CH_2, C_3), 53.2 (d, $^{2}J_{PC}$ = 7.3 Hz, CH_3, OMe), 53.6 (d, $^{2}J_{PC}$ = 7.2 Hz, CH_3, OMe), 60.6 (d, $^{1}J_{PC}$ = 159.3 Hz, CH, C_1), 122.6 (d, J_{PC} = 2.2 Hz, CH, Ar), 123.9 (d, J_{PC} = 3.3 Hz, CH, Ar), 127.1 (d, J_{PC} = 2.9 Hz, CH, Ar), 128.0 (d, J_{PC} = 3.0 Hz, CH, Ar), 136.3 (d, $^{3}J_{PC}$ = 3.9 Hz, C, C_{3a}), 141.7 (d, $^{2}J_{PC}$ = 7.2 Hz, C,

C_{7a}) ppm. HRMS (ESI^+, m/z) calcd for $C_{10}H_{14}NNaO_3P^+$ $(M+Na)^+$: 250.0604, found: 250.0606.

4.3. Synthesis of dimethyl [*N*-(allyloxycarbonyl)-1,3-dihydro-2*H*-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 aminophosphonate **2** (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 racemic carbamate **4a** as a white solid (70% isolated yield). R_f (80% EtOAc/hexane): 0.30. Mp: 74-76 ºC. ^{1}H-NMR ($CDCl_3$, 300.13 MHz) δ (dupplicated 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, $^3J_{PH}$ = 10.4 Hz) + 3.52 (d, $^3J_{PH}$ = 10.5 Hz, 3H, OMe)], [3.77* (d, $^3J_{PH}$ = 10.7 Hz) + 3.85 (d, $^3J_{PH}$ = 10.7 Hz, 3H, OMe)], 4.59-4.63 (m, 3H, H_3 + $H_{1'}$), 5.24 (d, $^2J_{HH}$ = 10.4 Hz, 1H, $H_{3'}$), 5.36 (dd, $^2J_{HH}$ = 10.4 Hz, $^3J_{HH}$ = 17.1 Hz, 1H, $H_{3'}$), [5.50* (d, $^2J_{PH}$ = 6.4 Hz) + 5.54 (d, $^2J_{PH}$ = 6.6 Hz, 1H, H_1)], 5.97 (ddt, $^3J_{HH}$ = 16.4, 10.6, 5.5 Hz, 1H, $H_{2'}$), 7.26-7.35 (m, 3H, Ar), 7.45-7.56 (m, 1H, Ar) ppm. ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ (dupplicated 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; doblet signals for the methoxy rotamers appears overlapped) [52.5 + 52.8* (CH_2, C_3)], 53.5-53.7 (m, $2CH_3$, OMe), 59.8 (d, $^1J_{PC}$ = 158.8 Hz, CH, C_1), [66.6 + 66.8* (CH_2, $C_{1'}$)], [117.9 + 118.3* (CH_2, $C_{3'}$)], [122.6 + 122.7* (CH, Ar)], 124.3 (CH, Ar), 127.7 (CH, Ar), 128.5 (CH, Ar), 132.7 (CH, $C_{2'}$), 134.1 (C, Ar), 137.9 (C, Ar), 154.7 (C, C=O) ppm. HRMS (ESI^+, m/z) calcd for $C_{15}H_{22}NNaO_5P^+$ $(M+Na)^+$: 334.0815, found: 334.0825.

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

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 aminophosphonate **2** (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), yielding the carbamate **4b** as a light yellow liquid (99% isolated yield). R_f (100% EtOAc): 0.49. IR (NaCl): 1707, 1411, 1359, 1282, 1031 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz) δ (dupplicated signals are observed for some protons and they have been shown in brackets; asterisks indicate those corresponding to the minor rotamer) [3.38* (d, $^3J_{PH}$ = 10.4 Hz) + 3.51 (d, $^3J_{PH}$ = 10.4 Hz, 3H, OMe)], [3.57* (d, $^3J_{PH}$ = 10.7 Hz) + 3.84 (d, $^3J_{PH}$ = 10.7 Hz, 3H, OMe)], 4.59-4.74 (m, 1H, H_3), 4.87-5.07 (m, 1H, H_3), 5.14-5.29 (m, 2H, Cbz), [5.49* (d, $^2J_{PH}$ = 6.5 Hz) + 5.56 (d, $^2J_{PH}$ = 5.9 Hz, 1H, H_1)], 7.19-7.54 (m, 9H, Ar) ppm. ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ (dupplicated 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) δ [52.5 + 52.8* (CH_2, C_3)], [53.3* (d, $^2J_{PC}$ = 6.7 Hz), 53.6 (d, $^2J_{PC}$ = 4.3 Hz, CH_3, OMe),], 53.6-53.8 (m, CH_3, OMe), 59.8 (d, $^1J_{PC}$ = 158.8 Hz, CH, C_1), [67.6 + 67.9* (CH_2, Cbz)], [122.5 (d, J_{PC} = 3.0 Hz) + 122.6* (d, J_{PC} = 3.1 Hz, CH, Ar)], [124.2* (d, J_{PC} = 3.1 Hz) + 124.3 (d, J_{PC} = 3.4 Hz, CH, Ar)], 127.6-127.8 (m, CH, Ar), 128.1 (CH, Ar), 128.3 (CH, Ar), 128.3-128.5 (m, CH, Ar), 128.6 (4CH, Cbz), [133.8 (d, J_{PC} = 4.0 Hz), 134.0* (d, J_{PC} = 3.5 Hz, C, Ar)], [136.2*, 136.4 (C, Cbz)], [137.7* (d, J_{PC} = 5.4 Hz), 137.8 (d, J_{PC} = 5.7 Hz, C, Ar)], [154.7*, 154.8 (C, C=O)] ppm. HRMS (ESI^+, m/z) calcd for $C_{18}H_{20}NNaO_5P^+$ $(M+Na)^+$: 384.0971, found: 384.0976.

4.5. General procedure for the lipase-catalyzed dynamic resolution of dimethyl (1,3-dihydro-2*H*-isoindol-1-yl)phosphonate, 2

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 ºC and 250 rpm for 47 h. The enzyme was removed by filtration, washed with CH_2Cl_2 (3 x 5 mL), and the solvents removed under reduce pressure to reach a 77% conversion value (^{1}H-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 amiophosphonate (*S*)-**2** (9% *ee*) when eluting with a 100% EtOAc eluent. $[\alpha]_{20}^{D}$ –58.3 (*c* 0.3, EtOH) for 96% *ee*.

4.6. General procedure for the synthesis of the Mosher derivative (*RS*,*R*)-6

The (*S*)-Mosher's acid chloride (10 µL, 0.055 mmol, 1.25 equiv.) and dry Et_3N (8 µL, 0.055 mmol, 1.25 equiv.) were successively added to a solution of racemic aminophosphonate **2** (10 mg, 0.044 mmol) in dry CH_2Cl_2 (0.5 mL) under inert atmosphere. The mixture was stirred for 10 h at room temperature and then CH_2Cl_2 (10 mL) was added. The solution washed with H_2O (2 x 10 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was analyzed by ^{1}H-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 allyloxycarbonyl group of (*R*)-dimethyl [*N*-(allyloxycarbonyl)-1,3-dihydro-2*H*-isoindol-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 PPh_3 (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 CH_2Cl_2 (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 CH_2Cl_2 (10 mL) was added to the solution, which was washed with water (2 x 10 mL). The organic layer was dried with Na_2SO_4, 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 allyloxycarbonyl group reaction (0.064 mmol for a theoretically 100% yield) in dry CH_2Cl_2 (0.7 mL) and under inert atmosphere, the (*S*)-Mosher's acid chloride (15 µL, 0.080 mmol, 1.25 equiv.) and dry Et_3N (11 µL, 0.080 mmol, 1.25 equiv.) were successively added. The mixture was stirred for 10 h at room temperature and then CH_2Cl_2 (10 mL) was added. The solution was washed with H_2O (2 x 10 mL), dried with Na_2SO_4, 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).

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