

Supporting Information

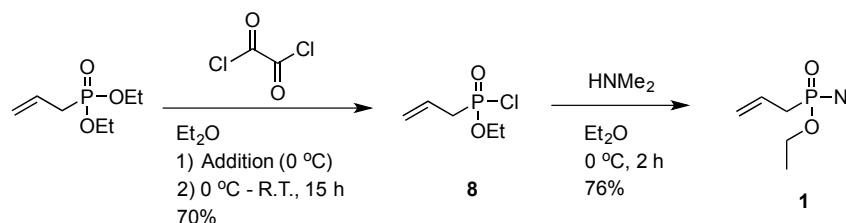
Lipase Active Site Covalent Anchoring of Rh(NHC) Catalysts: Towards Chemoselective Artificial Metalloenzymes

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SI-1. Materials and Methods.

Diethyl allylphosphonate, $[\text{Rh}(\mu\text{-Cl})(\text{cod})]_2$, 1,3-dimesityl-4,5-dihydroimidazolium chloride, potassium hexamethyldisilazide (KHMDs, 0.5 M in toluene), oxalyl chloride, $[\text{PdCl}_2(\text{dppf})]$, $\text{K}_3\text{PO}_4 \cdot \text{H}_2\text{O}$, *p*-nitrophenol, 9-Borabicyclo[3.3.1]nonane (9-BBN), dimethylamine, formic acid, acetophenone, methyl 2-acetamidoacrylate, 1,2-diphenylethane, *p*-nitrophenyl butyrate (pNPB) and candida antarctica lipase B (CalB) were purchased from Sigma Aldrich. Cutinase N172K was obtained from Novonordisk® with a calculated exact mass of 20603.1. Dry acetonitrile, diethyl ether, hexane and toluene were obtained from a MBraun MB SPS-800 solvent purification system; dichloromethane (DCM) and tetrahydrofuran (THF) were dried by distillation from CaCl_2 and sodium/benzophenone, respectively and stored over 4 Å molecular sieves. Tris(hydroxymethyl)aminomethane buffer (Tris-HCl) and $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ buffer were prepared in degassed Milli-Q water and stored in Schlenk flasks. Other solvents and reagents were purchased from commercial sources and used without further purification. ^1H , ^{13}C and ^{31}P NMR spectra were recorded at 298 K with a Varian AS 400 MHz NMR spectrometer at 400, 100 and 81 MHz, respectively. Chemical shifts are reported in ppm and referenced against the residual solvent signal. UV-Vis spectra were recorded with a Varian Cary50 Scan UV-Vis spectrometer. Chiral GC analyses were performed with a Perkin Elmer Gas Chromatograph AutoSystem XL equipped with an Agilent Cyclodex B 0.25 micron column and flame ionization detector. GC-MS analyses were performed with a Perkin Elmer gas chromatograph Clarus 680 equipped with a column PE Elite 5MS and coupled to a Perkin Elmer mass spectrometer Clarus SQ 8T with EI ionization. Electrospray Ionization (ESI-TOF) mass spectra of chemical products were recorded with a Waters LCT Premier XE KE317 Micromass Technologies spectrometer; mass spectrometry of protein and hybrid products (calculated as $[\text{M}]^{n+}=(\text{M}+n)/n$) were performed in positive ion mode using the previous apparatus and a Waters Synapt HD mass spectrometer (ESI-Q-IM-TOF) equipped with a Z-spray nanoelectrospray ionization source. Ultrafiltration dialysis of proteomic samples was performed with Vivaspins 6® tubes, 10,000 M.W. C.O. (PEG membrane). All reactions were carried out under standard Schlenk techniques and under inert conditions with N_2 .

SI-2. Synthesis of ethyl P-allyl-N,N-dimethylphosphoramidate 1.



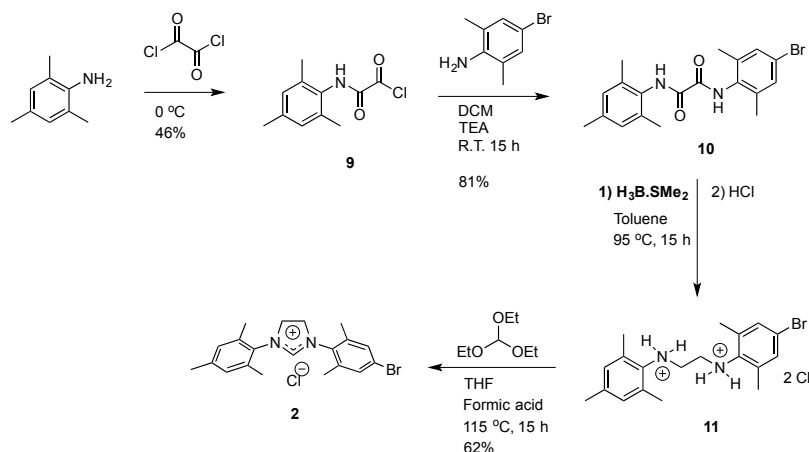
Scheme SI-2. Synthetic scheme towards phosphoramidate 1.^[1]

Synthetic procedure for chlorophosphate 8. Following an adaptation from C. A. Kruithof,^[1] Diethyl allylphosphonate (10 mL, 58 mmol) was dissolved in 40 mL dry DCM. Slowly oxalyl chloride (15 mL, 176 mmol) was added to the reaction mixture. The reaction was stirred for 21.5 hours. After which it was refluxed for 1 hour. All volatiles were removed by vacuum. ^1H NMR, CDCl_3 , 400 MHz, δ : 5.75 (m, CH, 1H), 5.28 (m, $=\text{CH}_2$, 2H), 4.26 (m, 4H, CH_2O), 2.90 (dd $^2J(\text{P})=20$ Hz, $^3J(\text{CH})=8$ Hz, PCH_2 , 2H), 1.33 (t

$^3J(\text{CH}_2)=8$, CH_3 , 3H); ^{13}C NMR, CDCl_3 , 100 MHz, δ : 125.2 (d $^2J(\text{P})=13$ Hz, CH), 122.1 (d $^3J(\text{P})=16$ Hz, $=\text{CH}_2$), 63.5 (d $^2J(\text{P})=9$, CH_2O), 38.9 (d $^1J(\text{P})=122$ Hz, PCH_2), 15.8 (d $^3J(\text{P})=7$ Hz, CH_3); ^{31}P , CDCl_3 , 81 MHz, δ : 39.2

Synthetic procedure for phosphonamidate 1.^[1] The residue of the previous reaction was dissolved in diethylether. Dimethylamine gas was condensed in a Schlenk submerged in liquid N_2 , about 10 mL (355 mmol) was added to the reaction mixture. The resulting reaction produced a large amount of gas and white solid. The reaction mixture was left stirring overnight, after which it was filtered and the residue was washed with dry diethylether (2x20 mL). The filtrate is concentrated on the rotational evaporator and the crude product is obtained as an orange liquid. The product was purified by reduced pressure distillation (Bp 50 °C / 0.04 mbar). The product is collected as a colourless oil. ^1H NMR, CDCl_3 , 400 MHz, δ : 5.76 (m, $=\text{CH}$, 1H), 5.10 (m, $=\text{CH}_2$, 2H), 3.90 (dm, $\text{CH}_2\text{-OP}$, 2H), 2.63 (d $^3J(\text{P})=8$ Hz, $\text{PN-(CH}_3)_2$, 6H), 2.5 (m, $p\text{-CH}_2$, 1H), 1.24 (t $^3J(\text{CH}_2)=6.8$ Hz, CH_3 , 3H); ^{13}C NMR, CDCl_3 , 100 MHz, δ : 128.2 (d $^2J(\text{P})=10$ Hz, $=\text{CH}_2$), 119.2 (d $^2J(\text{P})=13$ Hz, $=\text{CH}$), 59.4 (d $^2J(\text{P})=7$ Hz, PO-CH_2), 36.4 (d $^2J(\text{P})=4$ Hz, $\text{N(CH}_3)_2$), 31.1 (d $^1J(\text{P})=120$ Hz, $p\text{-CH}_2$), 16.2 (d $^3J(\text{P})=7$ Hz, CH_3); ^{31}P , CDCl_3 , 81 MHz, δ : 31.66.

SI-3. Synthesis of 1-mesityl-3-(4-bromo-2,5-dimethylphenyl)-4,5-dihydroimidazolinium chloride 4.



Scheme SI-3. Synthetic route towards bromo imidazolinium 4.

Synthetic procedure for anilide 9. Following a modification of a protocol previously described,^[2] oxalyl chloride (15 mL, 176 mmol) was cooled while stirring to 0 °C using an ice bath. 2,4,6-trimethylaniline (4.9 mL, 35 mmol) was dissolved in dry DCM (30 mL) and was slowly added. The resulting mixture was stirred overnight at room temperature after which the volatiles were removed under vacuum. Diethylether (100 mL) was added and the yellowish suspension filtered. The yellow filtrate was condensed under vacuum and the remaining solid was washed with hexanes. The suspension was filtered and the white solids collected and dried overnight under vacuum. ^1H NMR, CDCl_3 , 400 MHz, δ : 7.88 (bs, N-H, 1H), 6.86 (s, Ar-H, 2H), 2.22 (s, Ar- CH_3 , 3H), 2.12 (s, Ar- CH_3 , 6H); ^{13}C NMR, CDCl_3 , 100 MHz, δ : 167.8 (CClO), 151.4 (CNO), 137.3, 133.6, 128.3, 127.9 (Ar), 19.9, 17.2 (Ar-C).

Synthetic procedure for diamide 10. Following the synthesis of Gilbertson and Xu,^[3] the product of the previous reaction (2.5 g, 11 mmol) was dissolved in dry DCM (35 mL) and then cooled to 0 °C while stirring. To this a mixture of 4-bromo-2,5-dimethylaniline (2.3 g, 11 mmol), triethylamine (1.5 mL, 11 mmol) and dry DCM (10 mL) was slowly added after which the resulting reaction mixture was stirred overnight. Diethylether was added and the solids were collected by filtration. The solids were dissolved in DCM, and the resulting suspension was filtered yielding the first fraction of the product, which was washed with ether. The filtrate was washed with water, then dried over MgSO_4 . The solvent was removed and the residue was washed with a 10% DCM in diethylether solution. The suspension was filtered and the residue collected as the second fraction of the product. The fractions were combined and dried under vacuum. The

product was collected as a white solid. ^1H NMR, CDCl_3 , 400 MHz, δ : 8.69, 8.75 (bs, N-H, 1H), 7.21, 6.87 (s, Ar-H, 2H), 2.23 (s, Ar- CH_3 , 3H), 2.18, 2.16 (s, Ar- CH_3 , 6H); ^{13}C NMR, CDCl_3 , 100 MHz δ : 158.2, 157.9 (CNO), 137.7, 137.1, 134.0, 131.5, 131.2, 129.5, 121.4 (Ar), 20.9, 18.3 (Ar- CH_3).

Synthetic procedure for diammonium salt 11.^[3] The previous product (2.5 g, 6.5 mmol) was suspended in 50 mL dry and degassed toluene. Borane methyl sulphide complex (2.8 mL, 30 mmol) was added. The reaction mixture was stirred overnight at 95 °C, yielding a clear yellow solution. After the reaction mixture cooled down to room temperature a 3M HCl solution was added. The solution started to bubble and solids precipitate. HCl was added until the pH was 2. The mixture was then left stirring for 48 hours, after which it was filtered and the residue was first washed with water, then with a 10% acetone in diethylether solution. The product was used directly for the next step. ^1H NMR, DMSO, 400 MHz, δ : 7.15, 6.89 (s, Ar-H, 2H) 4.30 (bs $\text{CH}_2/\text{NH}_2^+$) 2.43, 2.23, 2.06 (s, Ar- CH_3); ^{13}C NMR, CDCl_3 , 100 MHz, δ : 143.1, 138.7, 133.1, 132.2, 131.5, 130.9, 115.2 (Ar), 50.1, 44.3, (CH_2) 20.7, 18.7, 18.2 (Ar- CH_3).

Synthetic procedure for bromo imidazolinium salt 2. The previous product was dissolved in triethyl orthoformate (20 mL, 152 mmol), 3 drops of concentrated formic acid were added and the suspension was stirred at 125 °C, at which it becomes a clear yellowish brown solution, then the reaction is left to cool. A large amount of hexanes were added (100 - 150 mL) and the mixture was stirred for 3 hours. The resulting suspension was filtered and the residue washed with more hexanes. The product was collected as a white solid. To our knowledge, there is no previous report of this compound. ^1H NMR, DMSO, 400 MHz, δ : 9.29 (s, $\text{N}_2\text{C-H}$, 1H), 7.52 (s, Ar-H, 2H), 7.06 (s, Ar-H, 2H), 4.49 (CH_2 , 2H), 3.41 (m, CH_2 , 2H) 2.39 (s, Ar- CH_3 , 6H), 2.34 (s, Ar- CH_3 , 6H), 2.27 (s, Ar- CH_3 , 3H); ^{13}C NMR, CDCl_3 , 100 MHz, δ : 160.7 (N_2CH), 140.0, 139.1, 135.8, 133.3, 131.8, 131.3, 129.9 123.3 (Ar), 56.4, 51.4, (CH_2CH_2), 21.0, 19.0, 17.6 (Ar- CH_3).

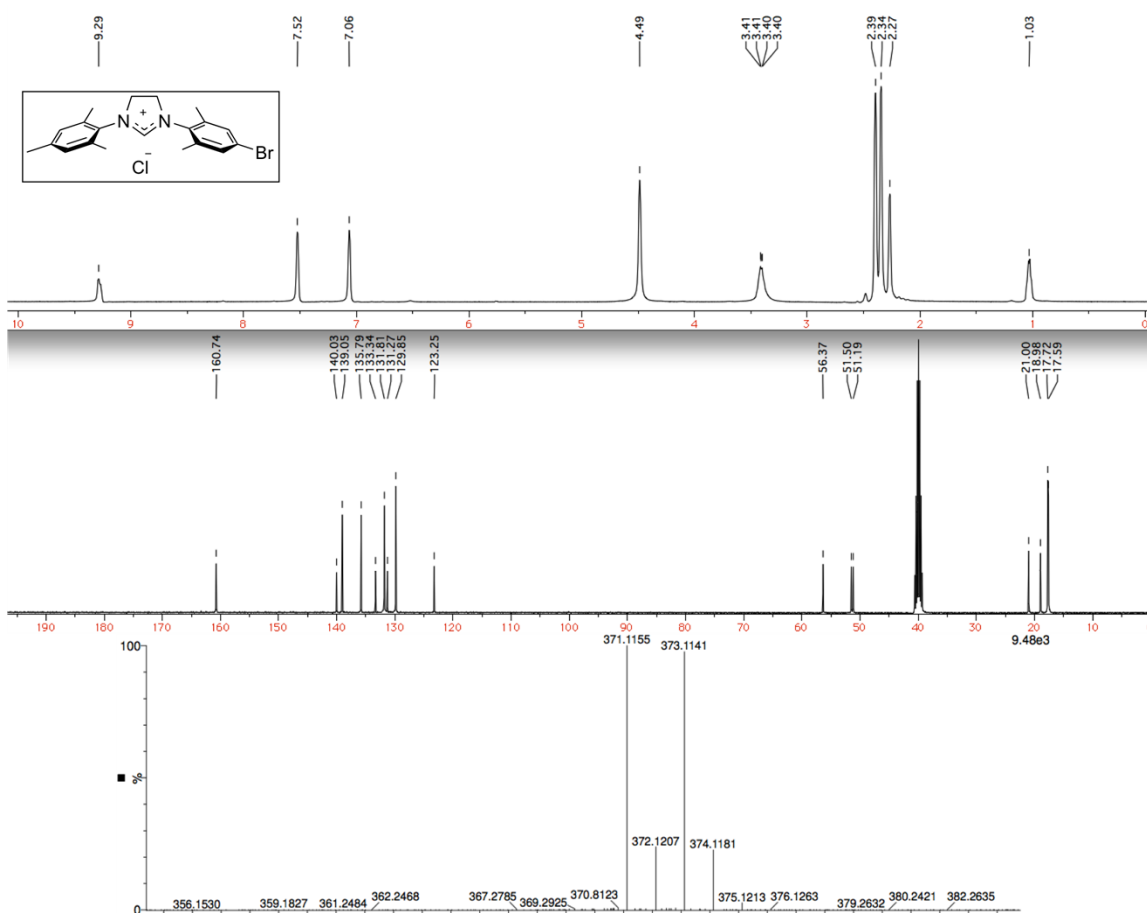


Figure SI-3. Spectra of compound 2. From top to bottom: NMR ^1H , ^{13}C and ^{31}P , ESI-MS (Calc.: 371.1117, found: 371.1155).

SI-4. Synthesis of ethyl *P*-(3-(4-(3-mesityl-4,5-dihydroimidazolinium)-3,5-dimethylphenyl)propyl)-*N,N*-dimethylphosphonamidate chloride **3.** In the glovebox, [9-BBN]₂ (0.15 g, 0.63 mmol of dimer) was weighed, dissolved in THF (15 mL) and added to a solution of the allyl phosphonamidate **1** (0.19 g, 1.05 mmol) also in THF (5 mL) in a Schlenk at room temperature and stirred for 20 h. The reaction mixture was concentrated in vacuo, closed and removed from the glovebox. To this hydroboronated phosphonamidate, a solution of the bromo imidazolinium salt **2** (0.39 g, 0.95 mmol) and [Pd(dppf)Cl₂]-CH₂Cl₂ catalyst (54 mg, 67 μ mol) in DMF (38 mL) was added, followed by K₃PO₄·H₂O solid (0.22 g, 1.06 mmol). The mixture was stirred under N₂ and 2 mL of degassed water was added. A reflux condenser adapted and the reaction was left stirring at 100 C for 21 h. At the end of this time, the reaction was cooled down to room temperature and concentrated under rotary evaporator and then under high vacuum (10⁻⁶ mbar at 50 C for 1.5 h). The residue was redissolved in DCM (15 mL) and the solids filtered off. The product was purified by column chromatography using silica gel with CH₂Cl₂/MeOH (9:1, R_f = 0.5) eluent as a dark yellow very viscous oil (288 mg, 60%). ¹H NMR, CDCl₃, 400 MHz, δ : 8.90 (s, NCHN, 1H), 6.94 (s, ArH, 4H), 4.62 (s, NCH₂CH₂N, 4H), 3.90 (dm, CH₂O, 2H), 2.64 (d ³J(P)=8 Hz, NCH₃, 6H), 2.61 (m, ArCH₂, 2H), 2.39, 2.38 (s, ArCH₃, 12H), 2.27 (s, ArCH₃, 3H), 1.84 (m, CH₂P, 2H), 1.63 (m, CH₂CH₂P, 2H), 1.25 (t, ³J=7 Hz, CH₂CH₃, 3H); ¹³C NMR, CDCl₃, 100 MHz, δ : 159.2 (NCHN), 144.1, 140.7, 135.2, 134.9, 130.6, 130.1, 130.0, 129.5 (Ar), 59.2 (d ²J(CH₂)=6 Hz, CH₂O), 52.3 (NCH₂CH₂N), 36.1 (m, NCH₃), 24.1 (d, ¹J(C-P)=10 Hz, CH₂P), 23.7, (d, CH₂CH₂P), 23.6 (ArCH₂), 21.0, 18.2 (ArCH₃), 16.3 (CH₂CH₃); ³¹P, CDCl₃, 81 MHz, δ : 36.22. ESI-MS [M-Cl]⁺ calc.: 470.2931. Found: 470.2970.

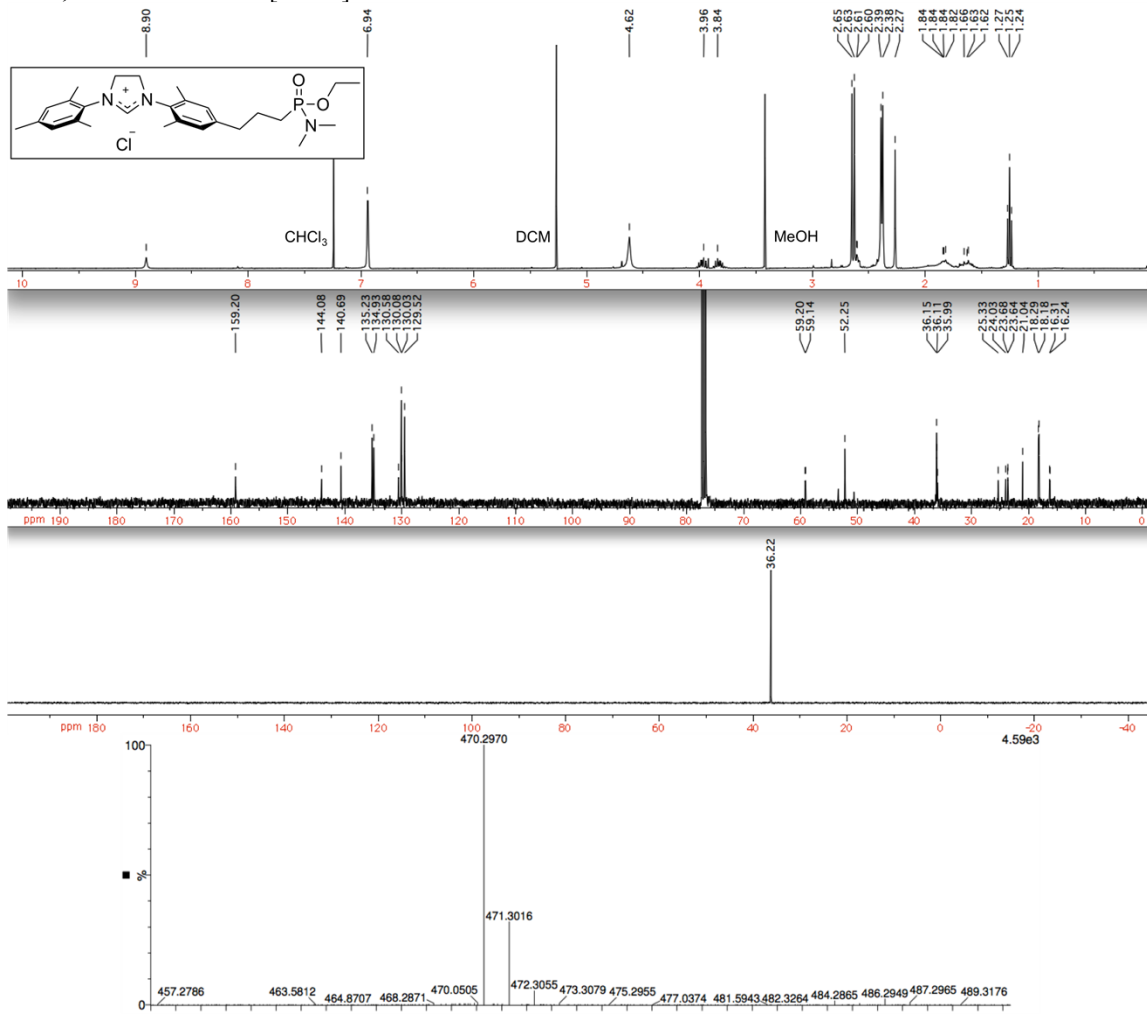


Figure SI-4. Spectra of compound **3**. From top to bottom: NMR ¹H, ¹³C and ³¹P, ESI-MS (Calc.: 470.2931, found: 470.2970).

SI-5. Synthesis of RhCl(cod)(NHC)phosphonamidate 4. The phosphonamidate-SIMes product (**3**) (42.6 mg, 0.084 mmol) was placed in a Schlenk flask and brought into the glovebox. Toluene (10 mL) was added and the mixture stirred, followed by slow addition of KHMDS 0.5 M in toluene (0.18 mL, 0.09 mmol); the heterogeneous mixture was stirred at room temperature for a total of 20 h and to the new dark orange solution, [Rh(μ -Cl)(cod)]₂ (17.8 mg, 0.036 mmol) in toluene (5 mL) was slowly added. The mixture was stirred for 0.5 h at room temperature and then for 2 h at 70 °C outside of the glovebox with a reflux condenser under N₂ atmosphere. After this time, the reaction was cooled down to about 10 °C filtered via cannula. The solvent was removed with vacuum and the product purified by column chromatography with silica and acetone as the eluent (*R_f* = 0.65). The product was obtained as a dark yellow to orange viscous oil (38.6 mg, 75%). ¹H NMR, CD₂Cl₂, 400 MHz, δ : 7.03, 7.01, 7.00 (s, ArH, 4H), 4.48, 4.36 (s, CH_{cod}, 1H), 4.00 (m, CH₂CH₃, 2H), 3.84, 3.83(3x) (NCH₂CH₂N, 4H) 3.51, 3.45 (d, CH_{cod}, 2H), 2.69 (s, ArCH₂, 2H), 2.66, 2.63 (d, NCH₃, 6H), 2.56, 2.57 (d, ArCH₃), 2.34 (s, ArCH₃), 1.78 (m, CH₂P, 2H), 1.73 (m, CH₂CH₂P, 2H), 1.51, 1.49 (CH₂(cod), 2H), 1.27, 1.26 (t, CH₂CH₃, 6H), 1.22 (d, CH₂(cod), 2H); ¹³C NMR, CD₂Cl₂, 100 MHz, δ : 212.4, 212.2 (d, ¹J(C-Rh)=48 Hz, NCN), 141.3, 141.4, 138.4, 138.3, 138.2, 138.1, 138.0, 137.7, 137.7, 137.0, 136.5, 136.4, 135.6, 135.6, 135.4, 135.4, 129.5, 129.5, 129.1, 129.1, 129.0, 128.5, 128.4, 128.0, 127.9, 127.8 (Ar), 96.8, 96.7, 96.6, 96.2 (cod), 68.9, 68.7 (cod), 67.7, 67.6 (cod), 58.9 (d, POCH₂), 58.8 (d, POCH₂), 51.6, 51.5 (NCH₂CH₂N), 36.2, 35.8 (NCH₃), 32.5 (cod), 28.13 (cod), 25.3, 24.7 (d, CH₂P), 24.0 (CH₂CH₂P), 20.7, 20.5, 20.4, 18.26 (ArCH₃), 16.2, 16.1 (CH₂CH₃); ³¹P, CD₂Cl₂, 81 MHz, δ : 36.95. ESI-MS [M-Cl]⁺ calc.: 680.2847, found: 680.2859.

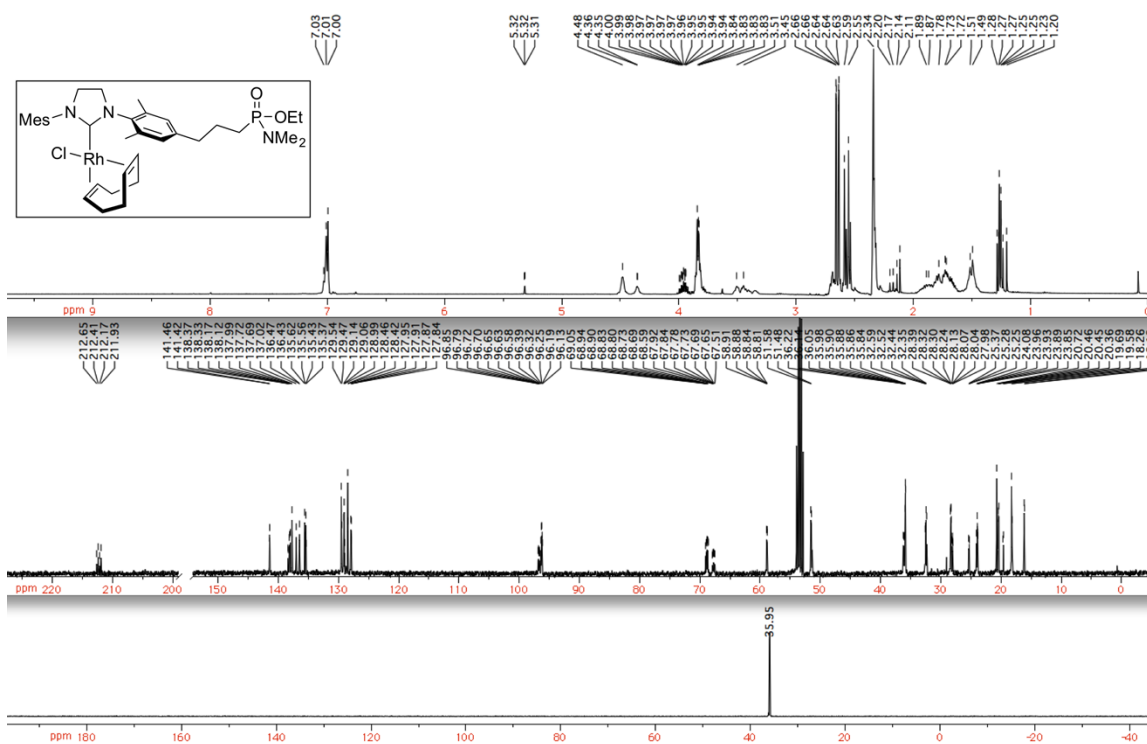


Figure SI-5.1. Spectra of compound 6. From top to bottom: NMR ¹H, ¹³C and ³¹P,

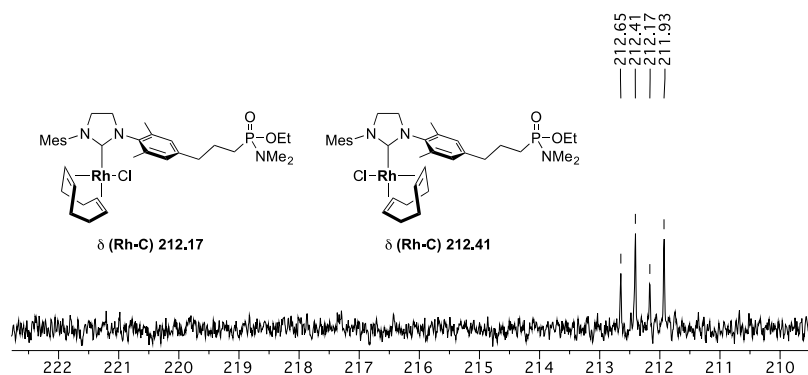


Figure SI-5.2. Low field ^{13}C NMR of **4** showing the Rh-C coupling of two isomers. For both isomers: $J_{\text{C-Rh}} = 48$ Hz.

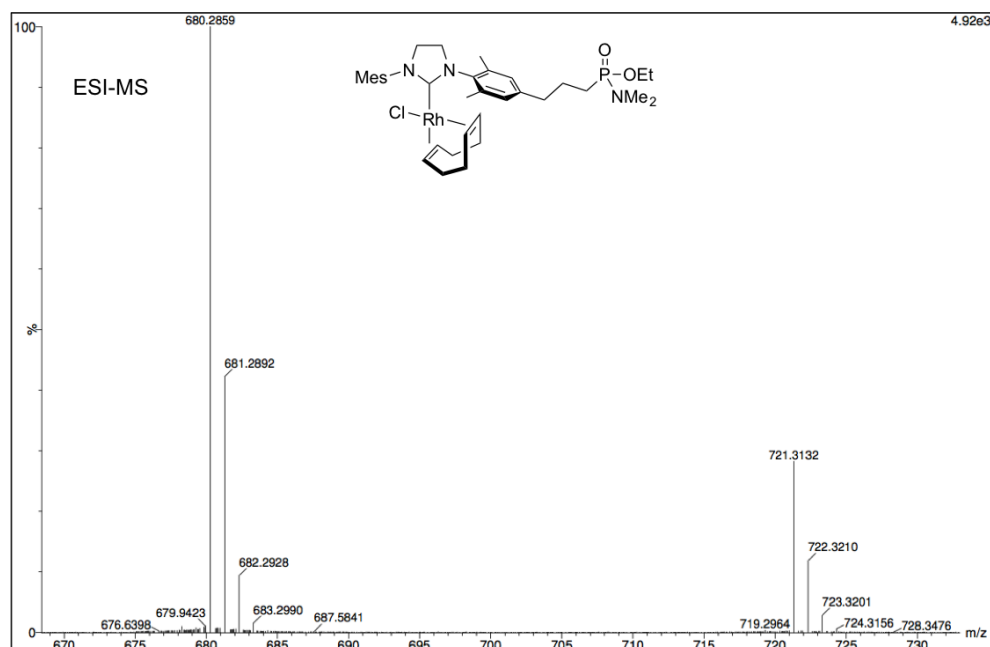


Figure SI-5.3. ESI-MS spectra of complex **4** ($[\text{M-Cl}]^+$ calc.: 680.2847, found: 680.2859; $[\text{M-Cl+MeCN}]^+$ calc.: 721.3118, found: 721.3132)

SI-6. Synthesis of lipase inhibitors.

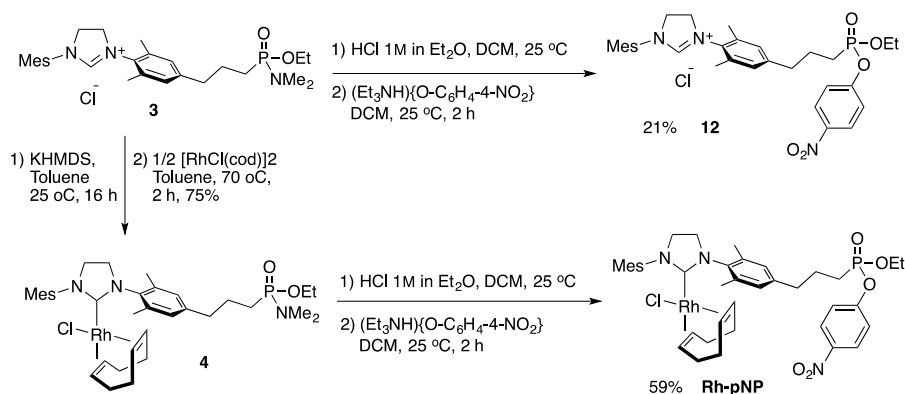


Figure SI-6. Synthetic route towards non-metallated and metallated lipase inhibitors **12** and **Rh-pNP**.

Synthesis of non-metallated inhibitor 12. To a solution of the phosphonamidate-SIMes product **3** (92 mg, 0.18 mmol) in DCM (5 mL), hydrogen chloride (0.9 mL, 1 M solution in diethyl ether, 0.9 mmol) was added and the mixture stirred for 2 h at room temperature. The reaction is concentrated in vacuo (an aliquot of the chloro phosphate product shows a single signal at 43.80 ppm in ^{31}P NMR) and DCM (5 mL) were added. A pre-stirred solution of *p*-nitrophenol (26 mg, 0.19 mmol) and triethylamine (0.07 mL, 0.5 mmol) in DCM (2 mL) is added to it and stirred for 3 h at room temperature. Solids are filtered off and the filtrate washed 1 time with aqueous K_2CO_3 1 M and saturated NaCl, collecting the organic fraction and concentrated to give an orange solid, a large portion of the product remains in the aqueous phase (14.7 mg, 21 %). ^1H NMR, CDCl_3 , 400 MHz, δ : 9.29 (s, NCHN, 1H), 8.19, 7.37 (d, $^3J=8$ Hz, $\text{ArH}_{(\text{pNP})}$, 4H), 6.93 (s, $\text{ArH}_{(\text{Mes})}$, 4H), 4.54 (s, $\text{NCH}_2\text{CH}_2\text{N}$, 4H), 4.17 (m, CH_2O , 2H), 2.66 (ArCH_2 , $^3J=8$ Hz, 2H), 2.38, 2.36 (ArCH_3 , 12H), 2.26 (ArCH_3 , 3H), 1.95 (m, CH_2P , 2H), 1.91 (b, $\text{CH}_2\text{CH}_2\text{P}$, 2H), 1.29 (t, $^3J=\text{CH}_2\text{CH}_3$, 3H); ^{13}C NMR, CDCl_3 , 100 MHz, δ : 160.1 (NCHN), 155.6, 143.2, 140.7, 135.5, 134.9, 130.9, 130.1, 129.4, 125.6, 121.0, 121.0 (Ar) 63.1 (d $^2J(\text{C-P})=7$ Hz, CH_2O), 51.8, 51.8 ($\text{NCH}_2\text{CH}_2\text{N}$), 29.7 (ArCH_2), 26.0 ($\text{CH}_2\text{CH}_2\text{P}$), 23.6 (d $^2J(\text{C-P})=50$ Hz, $\text{CH}_2\text{CH}_2\text{P}$), 21.0, 18.1, 18.0 (ArCH_3), 16.4 (CH_2CH_3); ^{31}P , CDCl_3 , 81 MHz, δ : 29.47. ESI-MS: Calc. Mass: $[\text{M}-\text{Cl}]$ 564.26. Found: 564.2628 (100%).

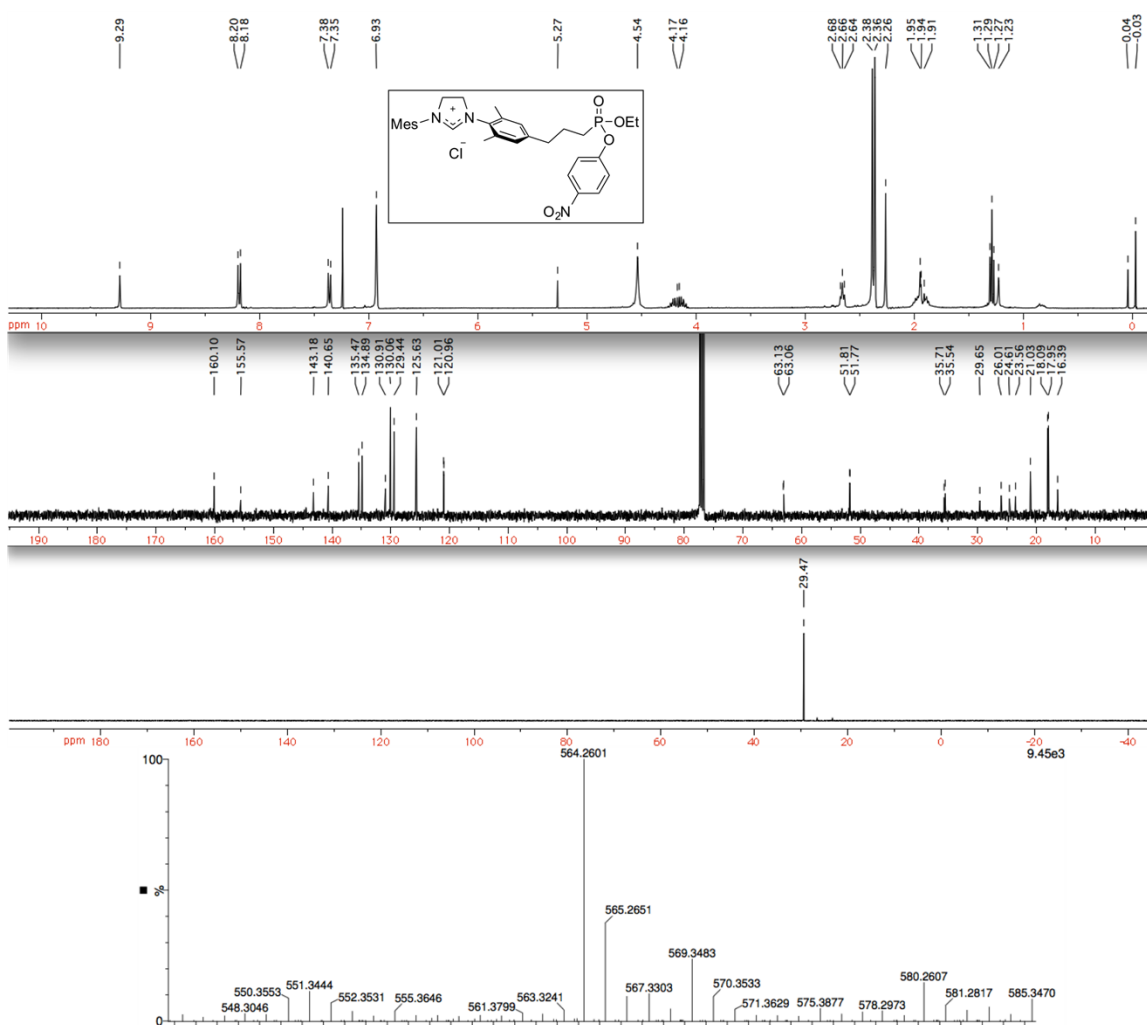


Figure SI-6.1. Spectra of inhibitor non-metallated lipase inhibitor **12**. From top to bottom: NMR ^1H , ^{13}C and ^{31}P , ESI-MS (Calc.: 564.2622, found: 564.2601).

Synthesis of Rh-inhibitor Rh-pNP. RhCl(cod)(NHC)phosphonamide **4** (24.8 mg, 0.035 mmol) was dissolved in DCM (5 mL), HCl (1 M in diethyl ether, 3.5 mL, 0.35 mmol), was added by syringe through septum and the mixture left stirring for 3 h at room temperature. After this time, the mixture was concentrated in vacuo under N₂, DCM (5mL) was added and a pre-stirred solution of *p*-nitrophenol (4.9 mg, 0.035 mmol) and triethylamine (20 μ L, 0.14 mmol) in DCM (3 mL) was added and stirred for 2 h at room temperature. The reaction is filtered via cannula and concentrated. The product is purified by silica column chromatography using acetone as the eluent (R_f = 0.78) yielding a dark yellow oil (16.8 mg, 59%). ¹H NMR, CD₂Cl₂, 400 MHz, δ : 7.00, 6.98 (s, ArH_{Mes}, 4H), 8.09 (³J=8 Hz, ArH_{pNP}, 4H), 4.35 (b, cod, 2H), 4.19 (m, CH₂CH₃, 2H), 3.86 x2 (s, NCH₂CH₂N, 4H), 3.48, 3.39 (cod, 4H), 2.74 (ArCH₂, 2H), 2.55, 2.52 (ArCH₃, 6H), 2.34, 2.33, 2.32 (ArCH₃, 6H), 2.04 (ArCH₃, 3H), 1.73 (m, CH₂P, 2H), 1.50 (m, CH₂CH₂P, 2H), 1.31, 1.30 (m, CH₂CH₃, 3H); ¹³C NMR, CD₂Cl₂, 100 MHz, δ : 211.61 (d, ¹J(C-Rh)=48 Hz, NCN), 162.5, 155.5, 155.4, 155.4, 144.7, 141.0, 140.5, 138.6, 138.0, 137.9, 137.2, 136.3, 136.0, 135.4, 129.6, 129.1, 128.6, 128.0, 127.9, 127.9, 125.9, 125.6, 121.0, 121.0, 115.6 (Ar), 97.1, 97.0, 96.9, 96.9 (cod), 68.6, 68.5, 68.2, 68.0 (cod), 63.6, 63.5, 63.4 (CH₂O), 51.6, 51.4 (NCH₂CH₂N), 35.7, 35.5 (cod), 32.7, 32.4 (cod), 28.2, 27.8 (CH₂CH₂P), 25.1 (d, ¹J(C-P)=140 Hz, CH₂P), 23.7 (ArCH₂), 20.7 (ArCH₃), 19.7, 19.5 (ArCH₃), 18.2, 18.1 (ArCH₃), 166.2, 16.1 (CH₂CH₃); ³¹P, CD₂Cl₂, 81 MHz, δ : 30.16. ESI-MS [M-Cl]⁺ calc.: 774.2538, found: 774.2538.

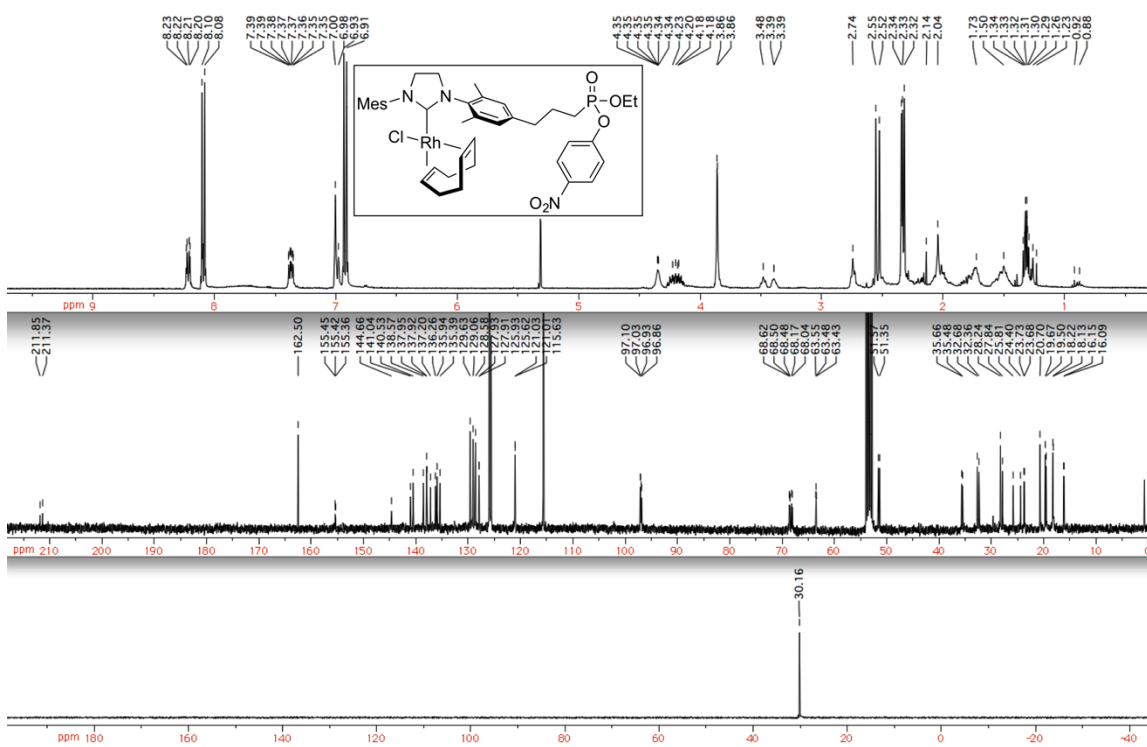


Figure SI-6.2. Spectra of inhibitor Rh-pNP. From top to bottom: NMR ¹H, ¹³C and ³¹P.

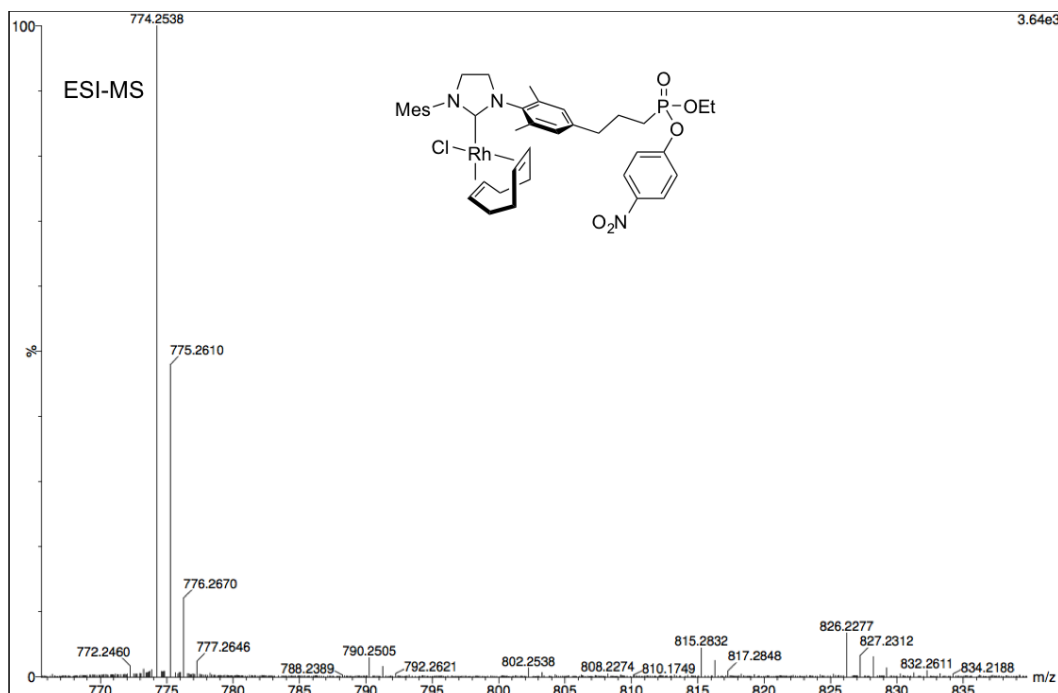


Figure SI-6.3. ESI-MS spectrum of target compound **Rh-pNP** ($[M-Cl]^+$ calc.: 774.2538, found: 774.2538; $[M-Cl+MeCN]^+$ calc.: 815.2809, found: 815.2832).

SI-7. Synthesis of $[Rh(SIMes)(cod)Cl]$ complex **5.** In the glovebox, to a stirred solution of 1,3-dimesityl-4,5-dihydroimidazolinium chloride (93 mg, 0.27 mmol) in toluene (15 mL), KHMDS 0.5 M in toluene (0.54 mL, 0.27 mmol) was slowly added, closed and left stirring at room temperature for 0.5 h. After this time and under stirring, a solution of $[Rh(\square-Cl)Cl(cod)]_2$ (66 mg, 0.13 mmol) in toluene (10 mL) was slowly added. The flask is closed and stirred at 70 °C for 2 h. After this time, the mixture was cooled down in an ice bath and the solids filtered off. The solvent was evaporated and the product purified by silica gel column chromatography using acetone as the eluent and collecting the dark yellow band. Evaporation of the solvent left an orange-red powder (99 mg, 66% yield). ESI-MS: $[M-Cl]^+$ calc.: 517.2090, found: 517.2090. NMR in agreement with previously reported spectra.^[4]

SI-8. Hybridization reactions through lipase inhibition.

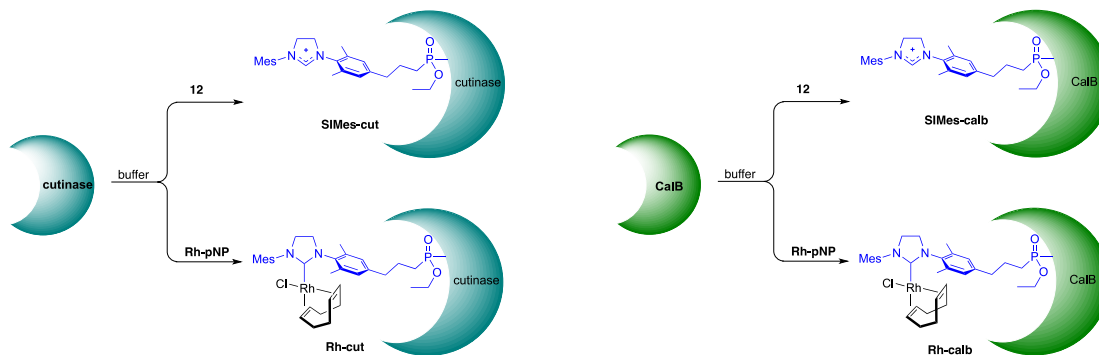


Figure SI-8.1. General inhibition reactions toward hybrids.

Synthesis of SIMes-cut and SIMes-calb hybrids. A stock solution of the lipase (cutinase or CalB) prepared from crystalline sample in Tris-HCl buffer (50 mM, pH 8.5) to give a protein concentration of 30.8 μM was treated with inhibitor **12** (4 mM) at room temperature, so that the initial ratio enzyme/inhibitor was 1:2 for **SIMes-cut** and 1:10 for **SIMes-calb**, final volume 2.5 mL and the buffer concentration 20 mM. The final concentration of proteomic content was 20 μM . (To determine the initial enzyme concentration, a parallel solution with the same amount of enzyme powder was dissolved in Milli-Q water and submitted to a nanodrop measurement of absorbance at 280 nm (extinction coefficient calculated from <http://web.expasy.org/protparam>, protein sequences obtained from uniprot.org database). The mixture was left to stir for additional 24 h at room temperature. Dialysis were conducted in membrane bags or by ultrafast filtration with 10 kDa M.W. C.O. membrane, restoring the volume each time with Milli-Q water and the last time with buffer in Milli-Q water. Hybrids were stored at -20 C.

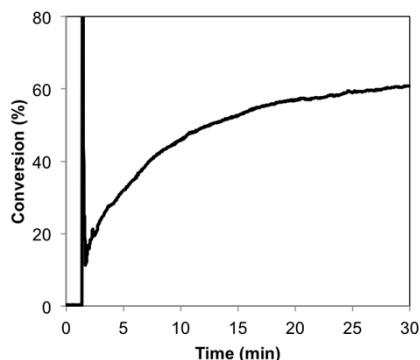
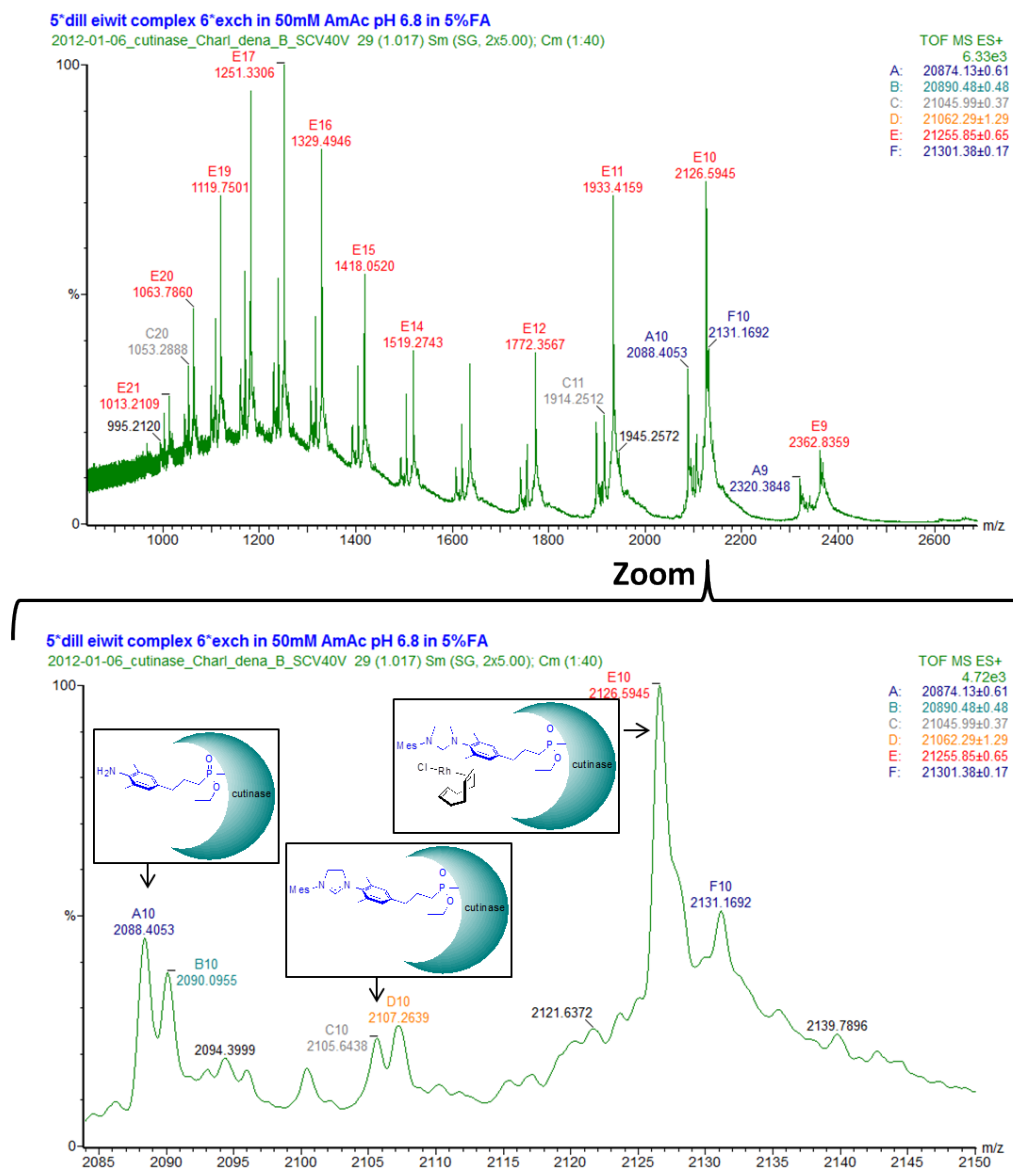


Figure SI-8.2. Release of *p*-nitrophenolate (pNP, $\lambda_{\text{max}} = 405 \text{ nm}$, $\epsilon = 18400 \text{ mol}^{-1}\text{cm}^{-1}$)^[5] during the inhibition of cutinase with **12**.

Synthesis of Rh-cut and Rh-calb hybrids. A similar procedure as for **SIMes-protein** hybrids was applied. To a stirred solution of cutinase or CalB (9.5 mL, 50 μM in degassed Tris-HCl or $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ buffer 50 mM, pH 8.5; concentrations determined by similar parallel experiments in Milli-Q water as explained above), inhibitor **Rh-pNP** (3 equivalents with respect to cutinase when buffer = Tris-HCl; or 10 equivalents when phosphate buffer was used and for the synthesis of **Rh-calb**) in degassed dichloromethane (DCM, 0.5 mL, solution was already yellow because of Rh complex) was slowly added via septum with syringe; the volume of DCM was 1/19 of the volume of buffer so that it accounted for 5%-vol. in the mixture. The reaction was stirred vigorously at 25 C for 24 h. Then the content is transferred to a 250 mL round bottom and placed in rotary evaporator with fast spin at 25 °C to eliminate the DCM. The solids are filtered off three times and the filtrate is transferred to Vivaspin 6® centrifugation tubes with peg membrane of 10 kDa M.W. C.O. and centrifuged $3 \times 1.5 \text{ h}$ at 3500 rpm for dialysis, restoring the volume each time with Milli-Q water and finally to 9.5 mL with buffer 50 mM. The resulting solutions were stored at -20 C under degassed vessels. For ESI-MS analyses, an aliquot of 0.5 mL is further dialysed and treated with formic acid for its denaturation before injection.



nH ⁺	21	20	19	18	17	16	15	14	13	12	11	10	9
Calc.	1013.06	1063.72	1119.70	1181.91	1251.43	1329.64	1418.29	1519.59	1636.49	1772.86	1934.03	2127.43	2363.81
Found	1013.21	1063.79	1119.75	1180	1251.33	1329.50	1418.05	1519.27	1635	1772.36	1933.42	2126.60	2362.84

Figure SI-8.3. Extended ESI-MS analysis of Rh-cut with the calculated and observed m/z values.

Residual activity of SIMes-calb. A cuvette was charged with *para*-nitrophenyl butyrate (pNPB, 1.14 mM, 1.35 mL, 1.5 μ mol), the absorbance at 405 nm was set to zero and a measurement of the production of pNP in function of time started (see Figure SI-6.1). The addition of reagents was as follows: At $t = 5$ min phosphate buffer pH 8.5 (1.35 mL of 100 mM); at $t = 15$ min SIMes-calb (0.3 mL of 50 μ M, 0.015 μ mol, equivalent to 1 mol-%); at $t = 30$ min unmodified CalB (0.3 mL of 50 μ M, 0.015 μ mol, equivalent to 1 mol-%).

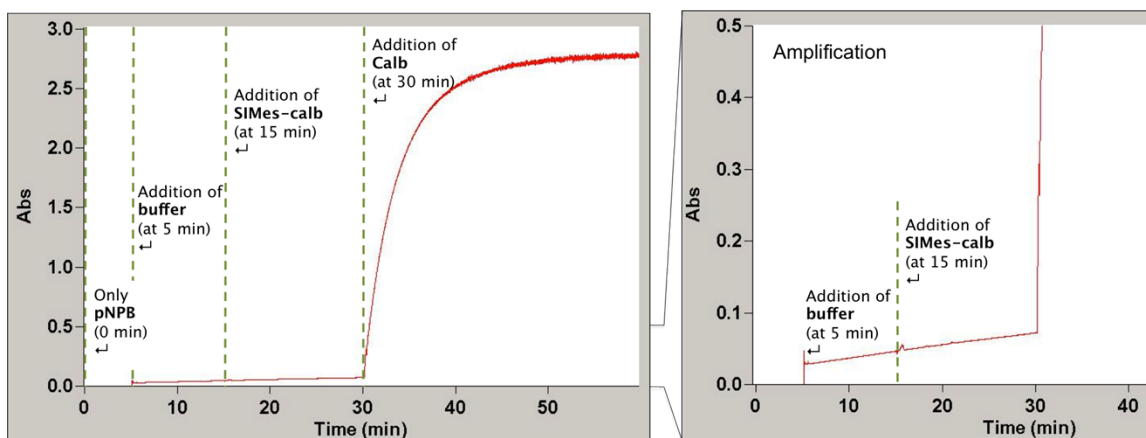


Figure SI-8.1. Monitoring of the release of pNP at sequential addition of CalB-containing species. At the addition of **SiMes-calb**, the very slow rate of hydrolysis of pNPB is equal to its hydrolysis in only buffer whereas the rate dramatically increases with the addition of unmodified CalB.

Residual activity of Rh-calb. With the absorbance set to zero the same as the previous procedure, a second cuvette was charged with only **Rh-calb** (0.3 mL of 50 μ M, 0.015 μ mol, equivalent to 1 mol-%) and phosphate buffer pH 8.5 (1.35 mL of 100 mM). The absorbance at 405 nm started in function of time (see Figure SI-6.2). The addition of reagents was as follows: At $t = 5$ min pNPB (1.35 mL of 1.14 mM, 1.5 μ mol), at $t = 15$ min unmodified CalB.

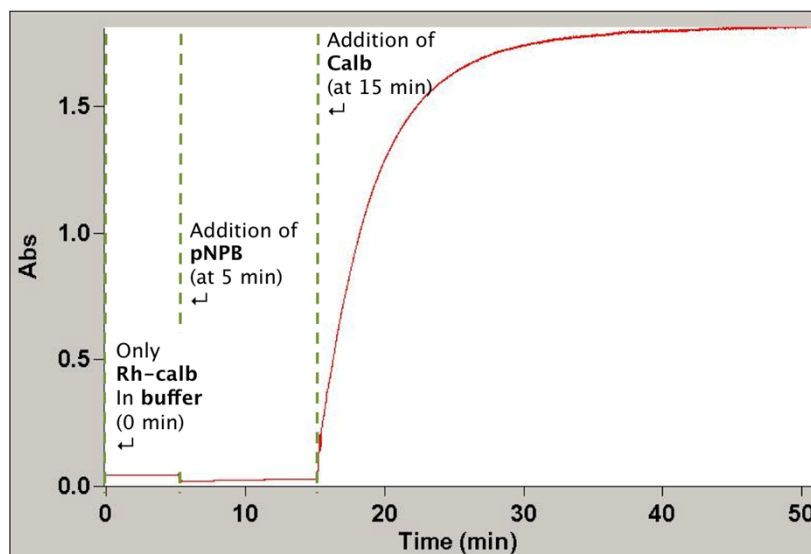


Figure SI-8.2. Monitoring of the release of pNP at sequential addition of CalB-containing species. pNPB in the presence of **Rh-calb** and buffer shows a very slow rate of hydrolysis comparable to the hydrolysis in only buffer (see Figure SI-6.1), whereas the rate dramatically increases when CalB is added. **Rh-calb** hybrid absorbs itself at 405 nm. The amount of pNPB is 100 times more than **Rh-calb**; even with higher concentration of the pNPB added, its addition increases the total volume of the system, therefore a decrease of absorbance is observed at 5 min.

SI-9. Catalysis.

Catalytic hydrogenation experiments using catalyst 5. In a typical procedure, a pressurization vessel was charged with substrate (1 mL stock solution of acetophenone and/or methyl 2-acetamidoacetate and 1,2-

diphenylethane as internal standard, 4.3 mM, 4.3 μ mol each in DCM) and catalyst **5** (1 mL of stock solution 0.21 mM, 0.215 μ mol, 5 mol-% in DCM). The solvent was evaporated and the vessel refilled with N₂ and DCM (0.25 mL). Buffer (4.3 mL, 50 mM) was added to the vessel together with a stirring bar and this was placed in an autoclave chamber. The system was flushed with a gentle stream of H₂ for 1 minute, then closed and pressurized under stirring to 40 bar of H₂. After the desired time of reaction, the chamber was opened. To this, DCM (3 x 5 mL) was added to extract the organic phase and then concentrated to ~ 0.05 mL and analyzed by chiral-GC and GC-MS, the latter to complement the determination of products identity correlating their ratios (and previous analysis of stock material). Column chromatography and NMR analysis of the reaction mixtures were incompatible due to the low amounts of analytes. Experiments with acetophenone, main text Table 1, Entry 4, but in presence of enzyme previously reacted with a dummy inhibitor resulted in equivalent yields of the alcohol.

Catalytic hydrogenation experiments using Rh-protein catalysts. In a typical procedure, a pressurization vessel was charged with substrate (1 mL stock solution of acetophenone and/or methyl 2-acetamidoacetate and 1,2-diphenylethane as internal standard, 4.3 mM, 4.3 μ mol each in DCM). The solvent was evaporated and the vessel refilled with N₂, DCM (0.25 mL). The hybrid (**Rh-cut** or **Rh-calb**, 4.3 mL, 50 μ M, 0.215 μ mol, equivalent to 5 mol-%) in buffer (50 mM) was added to the vessel together with a stirring bar and this was placed in an autoclave chamber. The system was flushed with a gentle stream of H₂ for 1 minute, then closed and pressurized under stirring to 40 bar of H₂. The resulting mixture has a clear colorless appearance, showing no denaturation of the protein material under the experimental conditions. The reaction and its analyses are equivalent to the proceedings described with catalyst **5**. Consistent data were obtained in repeated reactions and in reactions using hybrid samples that had been stored in a refrigerator over prolonged time (months). Table 1 in the manuscript shows the outcome of single experiments.

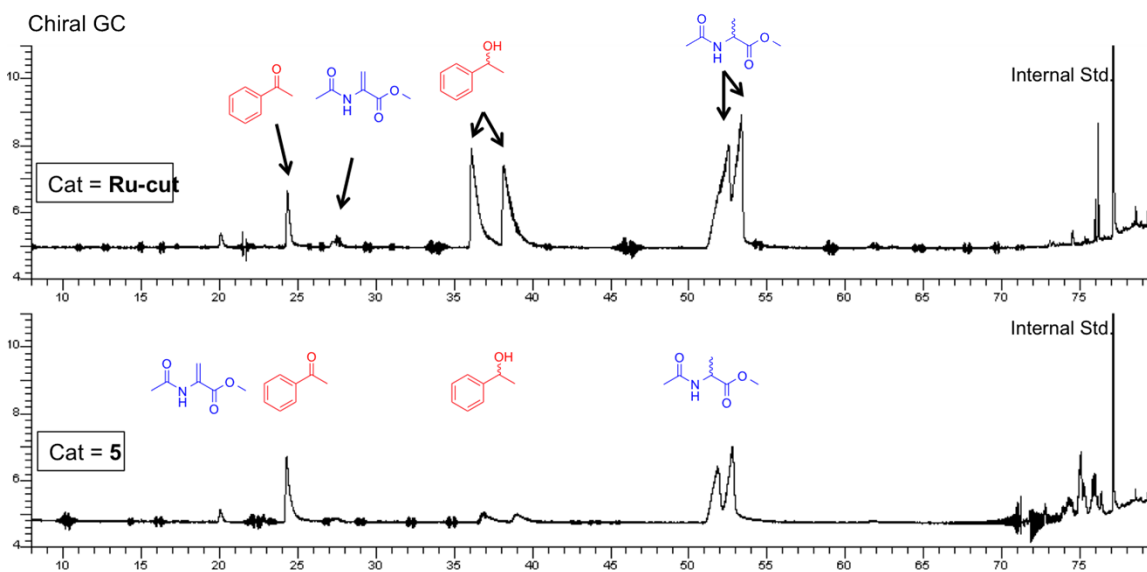


Figure SI-9.1. Resulting chromatogram of experiments for Table 1, entries 10 and 11; and Figure 2 of main manuscript.

SI-10. References

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