Design and Synthesis of Haptens for the Catalytic Antibody-Promoted Dynamic Kinetic Resolution of Oxazolin-5-ones

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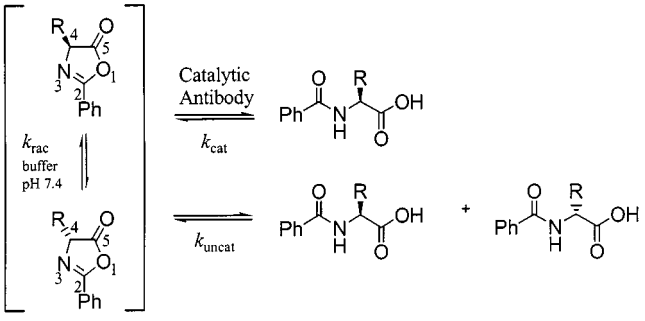
The synthesis of the first two haptens designed to elicit catalytic hydrolytic antibodies for the dynamic kinetic resolution of racemic 4-substituted 4*H*-oxazolin-5-ones is reported. A cyclic phosphinate and a 2,5-dihydro-1*H*-pyrrolium derivative were chosen as "first generation" haptens. The cyclic phosphinate was designed to mimic the higher energy transition state along the reaction coordinate for the hydrolytic process, while the dihydro-1*H*-pyrrolium group of the second hapten was selected to generate hydrolytic antibodies that might use a "bait and switch" mechanism. These two haptens were prepared successfully by use of a ring-closing metathesis reaction as the key step. This study is the first pilot investigation towards an antibody-promoted dynamic kinetic resolution and should allow the development of a new biocatalytic route to enantiomerically pure natural and unnatural amino acids.

Introduction

A considerable amount of effort has been devoted to the development of efficient chemical and biological catalysts for the synthesis of enantiomerically pure molecules. Recently, the principles and tools of organic chemistry have been used to exploit the immune system for the generation of antigen-induced catalysts. The specificity and, in some cases, the rates of the antibody-catalyzed reactions rival those of enzymatic systems. Abzymes are therefore a valuable addition to the pool of biocatalysts, especially for reactions that cannot be catalyzed by enzymes.[1]

In the synthesis of chiral intermediates, the resolution of racemic compounds with mediation by chiral reagents or biocatalysts has become a valuable tool.[2] In most cases, however, only one enantiomer of the intermediate is required in the synthesis, and the unwanted isomer has to be either discarded or racemized for recycling in the resolution process. Dynamic kinetic resolution (DKR) is one way of avoiding this problem.[3] In this process, one of the enantiomers is racemized in situ and thus provides fresh starting material for the resolution. Theoretically, a quantitative conversion of racemic starting material into one enantiomer of the product could be achievable; such processes have long been known in pure enzymatic or chemical synthesis.[3,4] Our goal here was to apply the concept of DKR to abzymatic synthesis.

We selected the enantioselective ring opening of 4-substituted 4*H*-oxazolin-5-ones as a model reaction (Scheme 1).



Scheme 1. Dynamic Kinetic Resolution of 4-substituted 2-phenyloxazolin-5-ones

This reaction is suitable for study of the feasibility of DKR with abzymes, because the substrates undergo spontaneous racemization under mild conditions, while the enantioselective hydrolysis of 4*H*-oxazol-5-one derivatives under catalysis by proteolytic enzymes and carboxyesterase has already been investigated by several groups as a new approach for the enzymatic asymmetric synthesis of α-amino acids. Initially, products of only modest optical purity were obtained, as the rates of enzyme-catalyzed ring opening were not considerably faster than those of competing nonenzymatic hydrolysis.[5] However, significant improvements were made by Sih et al. when they discovered that lipases are able to catalyze a highly enantioselective hydrolysis of 4-benzyl-2-phenyloxazolin-5-one (*ee* > 99%) in an aqueous buffer at pH 7.4. The same authors have reported

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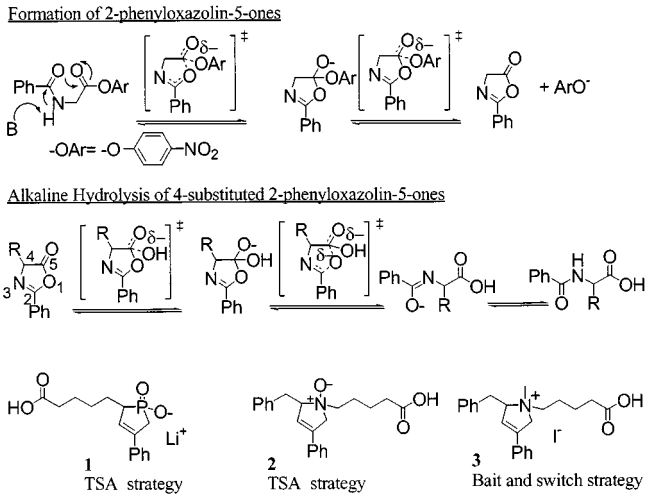
that lipase was also able to catalyze the enantioselective methanolysis of a variety of 4-substituted 2-phenyloxazolin-5-one derivatives in nonpolar organic solvents.[6] These results provided the main guidelines for the abzymatic DKR of oxazolinones.[7] In addition, antibody-catalyzed ester hydrolysis has been extensively studied and is well documented.[8] Here we report the design and the synthesis of the first two haptens necessary for the preparation of antibodies intended for catalysis of the enantioselective ring opening of 4*H*-oxazol-5-ones.

Results and Discussion

Hapten Design

With the aim of inducing catalytic antibodies for the alkaline cleavage of oxazolinones by immunization, we designed the two transition state analogues **1** and **2**, which would be both used as racemic compounds (Scheme 2). These analogues should be stable in biological systems and thus able to raise antibodies suitable for stabilization of the higher energy transition state on the reaction profile. On the basis of current mechanistic principles of organic chemistry, we postulated that the reaction should proceed through cyclic intermediates with a tetrahedral-like transition state possessing a partial negative charge distribution. The work of Williams[9] has established three discrete steps in the formation of an oxazolinone intermediate during alkaline hydrolysis of *N*-benzoylglycine *p*-nitrophenyl ester: (a) abstraction of the α-amido N−H proton, (b) addition of the oxygen of the α-amide anion to the ester carbonyl group, presumably to form a quasi-tetrahedral intermediate, and (c) breakdown of the intermediate, to release *p*-nitrophenolate anion and the oxazolin-5-one. Further studies by Matta et al.[9] on secondary and solvent kinetic isotope effects in this reaction are consistent with a transition state in which cyclization of the acyl moiety to a tetrahedral-like intermediate has occurred. Expulsion of *p*-nitrophenoxide from the tetrahedral-like intermediate is the rate-controlling step. As the formation of the oxazolinone is a reversible process, we anticipated that the rate-controlling step for the ring opening should be the attack of the nucleophile. Accordingly, the five-membered heterocycles **1** and **2** were chosen as first generation transition state analogues (TSAs) because they should mimic the geometric and electronic characteristics of the first step of the process.[10] To ensure the stability of the haptens, the endocyclic nitrogen and oxygen atoms were replaced by carbon atoms. This replacement was judged acceptable since the endocyclic double bond was preserved in both analogues. The position of attachment of the linker to the hapten **1** was chosen so as to allow broad substrate specificity.[11] In contrast, the TSA **2**, with the linker attached at the nitrogen atom, should permit study of the cleavage of 4-benzyl-2-phenyloxazolin-5-ones with a series of different nucleophiles. It was anticipated that product inhibition would be avoided, because the acyclic products should easily be released from the binding site of antibodies obtained by immunization with cyclic haptens. Regarding electronic features, a phosphinate function and a *N*-oxide group were chosen to mimic the anionic character of the transition state.[12]



Scheme 2. Haptens **1**, **2**, and **3** designed for the alkaline hydrolysis of 4-substituted 2-phenyloxazolin-5-ones

Unfortunately, it was never possible to obtain compound **2**, because the last step of its synthesis − the oxidation of the tertiary amine function into the corresponding *N*-oxide − only produced complex reaction mixtures. Consequently, we partially reconsidered our strategy and turned to an alternative approach to hapten design, with an attempt to generate antibodies that might use a "bait and switch" mechanism instead.[13] The incorporation of the highly polar ammonium group in hapten **3** should elicit complementary carboxylate groups in the antibody binding pocket, which would act as a general base. This "bait and switch" strategy has been shown to be superior in some cases to the more conventional approach of transition state mimicry.[9c]

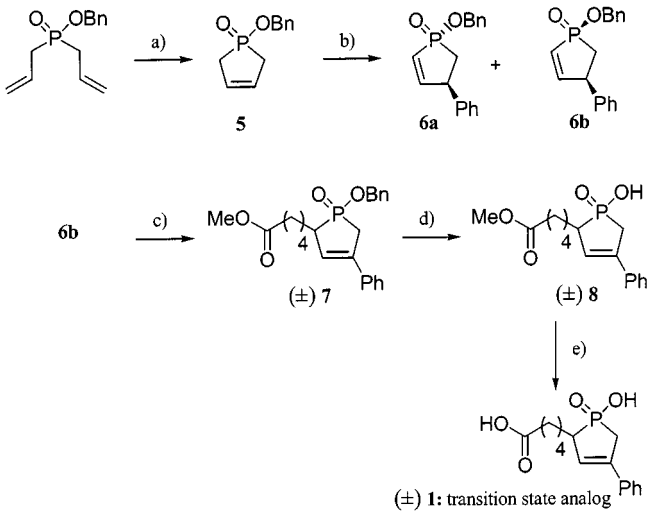
Synthesis

In the retrosynthetic analysis of heterocycles (±)-**1** and (±)-**3** (derived from **2**), the endocyclic double bond would derive from ring-closing metathesis (RCM),[14] an efficient method for the preparation of functionalized heterocycles from acyclic diene precursors.

Synthesis of TSA 1

RCM of allyl(2-phenylallyl)phosphinic acid should result directly in an advanced synthetic intermediate for the preparation of TSA **1**. However, we found that the RCM of diallylphosphinic acid, in contrast to RCM reactions on dienes including a phosphinate group, could not be achieved either with the Grubbs Ru-alkylidene **4**[15] or with the Schrock Mo-alkylidene.[16] RCM of allyl(2-phenylallyl)phosphinic acid benzyl ester was also not feasible with the Ru-catalyst **4**; only starting material was recovered. Consequently, we started our synthesis with 1-benzyloxy-3-phospholene 1-oxide **5** (Scheme 3), which was prepared by

RCM of the corresponding acyclic phosphinate diene with the aid of the Grubbs catalyst **4**.[17]



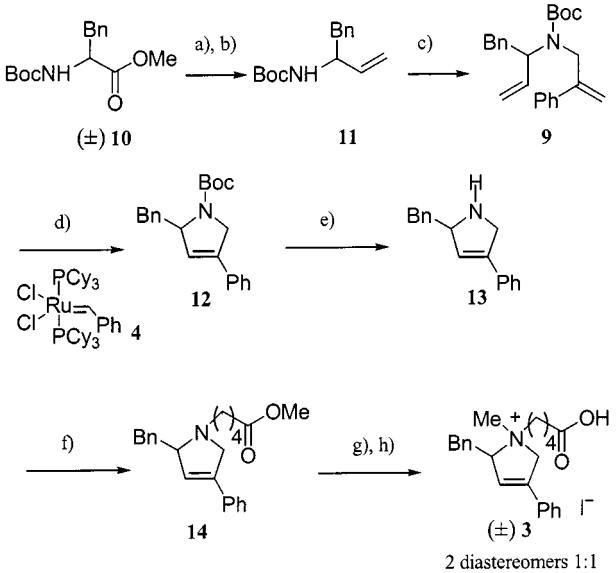
Scheme 3. Synthesis of the transition state analogue **1**: a) 2 mol% Grubbs catalyst **4**, CH_2Cl_2, reflux, 80%; b) Ph−N_2^+ BF_4^-, $Pd(OAc)_2$, MeOH, 50 °C, 75%; c) LDA, TMEDA, −78 °C, THF, I−$(CH_2)_4$−COOMe, 35%; d) TMSBr, CH_2Cl_2, room temp., 83%; e) 1 M LiOH, dioxane, 56%

The phenyl group was introduced into position by means of a Heck coupling.[18] After several attempts with iodobenzene and phenyl triflate, giving only low yields of product, we found that treatment of compound **5** with 1.5 equivalents of phenyldiazonium tetrafluoroborate and 3 mol% of $Pd(OAc)_2$ at 50 °C in MeOH afforded the expected products **6a** and **6b** in satisfactory yield (75%) after only 45 minutes.[19] Under these mild conditions, no double bond isomerization caused by the *syn* elimination and readdition of Pd−H species was observed. The reaction provided the two diastereomers **6a** and **6b** in a ratio of 6:4. These two compounds could be cleanly separated by column chromatography and fully characterized. The relative *syn* and *anti* stereochemistry of the two chiral centers (the phosphorus center and the benzylic carbon) in compounds **6a** and **6b** was assigned with the aid of bidimensional NOESY experiments. Correlations of the two hydrogens positioned α to the phosphinate group with hydrogens in close spatial relationships allowed us to reach conclusions on the spatial relationship of the benzyloxy and phenyl groups. Indeed, for diastereomer **6b**, two spatial interactions were detected for one of these hydrogens. Two cross-peaks with the benzylic proton of the phosphinate moiety and with the *ortho* proton of the phenyl group located β to the phosphinate group were observed. Moreover, no spatial interactions could be detected for the other hydrogen positioned α to the phosphinate group. These results are consistent with a stereoisomer in which the phenyl and the benzyl groups are in a *syn* relationship.

The introduction of the linker was achieved by the deprotonation of diastereomer **6b** with LDA in the presence of TMEDA, followed by alkylation with methyl 5-iodovalerate. This reaction afforded compound **7** as a single diastereomer, in a chemical yield of 35%. Under these conditions, alkylation occurred regioselectively at the position α to the phosphinate group. It should be noted that diastereomer **6a** was unreactive under these conditions. Deprotection of compound **7** was achieved in two steps. In the first step, the phosphinic acid **8** was released in a chemical yield of 83% after treatment with TMSBr[20] in dichloromethane. In the second step, LiOH in dioxane was used to saponify the methyl ester group to afford the desired TSA (±)-**1** as a white solid in 56% yield after recrystallization from a mixture of methanol and dichloromethane.

Synthesis of the Hapten 3

The RCM of the diene **9** should afford an advanced intermediate for the synthesis of the hapten **3** in one step (Scheme 4). We demonstrated that, unlike the RCM of allyl(2-phenylallyl)phosphinic acid benzyl ester, that of the phenyl-substituted 4-aza-1,6-diene **9** was possible, and afforded the desired cyclic product in 83% yield.[21] Consequently, diene **9** was prepared from the *N*-protected D,L-phenylalanine methyl ester **10** by a sequence described in the literature.[22] The α-ester group of **10** was first reduced with diisobutylaluminium hydride to provide an intermediate aluminoxy acetal, which, on treatment with the appropriate Wittig reagent, afforded the allylamine **11** in 35% overall yield. The 4-aza-1,6-diene **9** was obtained in 92% yield from compound **11** by alkylation with 3-bromo-2-phenylprop-1-ene in the presence of NaH. The RCM of diene **9** was accomplished in benzene at 60 °C in the presence of 2% of Ru−alkylidene **4** (added portionwise) to afford compound **12** in 83% yield. The presence of rotamers in compounds **11** and **12** was confirmed by NMR experiments at higher temperatures, at which coalescence of the signals



Scheme 4. Synthesis of hapten **3**: a) Dibal-H, toluene, −78 °C; b) PPh_3=CH_2, −78 °C to 50 °C, 35%; c) NaH, CH_2=$CPhCH_2Br$, DMF, 0 °C to room temp., 92%; d) 2 mol% Grubbs catalyst **4**, benzene, 60 °C, 83%; e) TFA, CH_2Cl_2, room temp., 98%; f) $Br(CH_2)_4COOMe$, K_2CO_3, CH_3CN reflux 85%; g) 1 M LiOH, dioxane, 52%; h) MeI, acetone, room temp., 100%

was observed. The *tert*-butyl carbamate was cleaved almost quantitatively by a solution of 20% trifluoroacetic acid in dichloromethane, to give compound **13** as an oil. The linker was introduced by *N*-alkylation with methyl 5-bromovalerate in acetonitrile in the presence of K_2CO_3, to give compound **14** in a chemical yield of 85%. Saponification of the ester group of **14** with LiOH, followed by quaternization of the nitrogen in the presence of methyl iodide, afforded compound (±)-**3** as a 1:1 mixture of the two diastereomers.

Conclusion

The design and synthesis of the first two haptens for the catalytic antibody-promoted DKR of 4*H*-oxazol-5-ones have been reported. Two strategies − transition state mimicry and a "bait and switch" strategy − were adopted for the hapten design. Straightforward syntheses with RCM reactions as their key steps were developed. The production of monoclonal antibodies against BSA and KLH conjugates of these haptens and their characterization for catalysis will be described separately.

Experimental Section

General Remarks: When appropriate, reactions were carried out in dried glassware under inert argon atmosphere. All solvents were dried by standard methods before use. THF was distilled from sodium/benzophenone under argon atmosphere, dichloromethane and acetonitrile from calcium hydride under argon atmosphere. Methanol, triethylamine, and benzene were kept dry over sodium under argon; DMF, TMEDA, and acetone over molecular sieves. Commercially available starting materials were used without further purification. TLC: commercially precoated Merck plates $60F_{254}$ silica gel. Viewing was accomplished with UV light and phosphomolybdic acid solution. Flash column chromatography were performed with Merck Si 60 silica gel (40−63 μm) with ethyl acetate/hexane mixture as eluent. Melting points were determined on a Reichert Jung apparatus. ^{1}H, ^{13}C, and ^{31}P spectra were recorded on Bruker AC 200 or DPX 300 machines and IR spectra on a Perkin−Elmer 1600 FT-IR. Mass spectra were performed by A. Valleix (CEA Saclay), HMRS by P. Guenot (Université de Rennes I), and elemental analysis by the Service Central d'Analyses CNRS (Gif sur Yvette).

1-Benzyloxy-4-phenyl-2-phospholene 1-Oxide (6a and 6b): A solution of compound **5**[14] (1.2 g, 5.8 mmol), phenyldiazonium tetrafluoroborate (1.7 g, 8.7 mmol), and $Pd(OAc)_2$ (40 mg, 0.17 mmol) in dry methanol (20 mL) was heated at 50 °C. After 45 min, the reaction mixture was treated with a saturated aqueous solution of NH_4Cl (10 mL) and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with water (20 mL), dried with $MgSO_4$, filtered, and concentrated under vacuum. The oil was purified by silica gel chromatography (hexane/EtOAc, 4:1) to afford the two diastereomers **6a** (740 mg) and **6b** (500 mg) as white solids (overall yield 1.24 g, 75%).

Compound 6a: R_f = 0.64 (EtOAc); m.p. 60 °C (hexane/EtOAc). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.45−7.16 (m, 10 H), 6.92 (ddd, *J* = 50.9, *J* = 8.3, *J* = 2.2, 1 H), 6.23 (ddd, *J* = 22.6, *J* = 8.3, *J* = 2.6, 1 H), 5.16 (dd, *J* = 12.0, *J* = 9.6, 1 H), 5.12 (dd, *J* = 12.0, *J* = 9.5, 1 H), 3.94 (m, 1 H), 2.38 (ddd, *J* = 15.4, *J* = 13.7, *J* = 8.3, 1 H), 1.84 (ddd, *J* = 15.8, *J* = 13.7, *J* = 5.3, 1 H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 154.7 (d, *J* = 27.6), 142.4 (d, *J* = 8.7), 136.5 (d, *J* = 5.8), 129.1, 128.7, 128.6, 128.3, 127.4, 127.2, 124.2 (d, *J* = 119.1), 66.9 (d, *J* = 5.8), 45.3 (d, *J* = 14.5), 31.4 (d, *J* = 93.0). ^{31}P NMR ($CDCl_3$, 121.5 MHz): δ = 73.5. IR (KBr): ν̃ = 3031, 1455, 1323, 1225, 999, 876, 733 cm^{-1}. MS (CI/NH_3): *m/z* (%) = 302 (100) $[M + NH_4]^+$, 285 (41) $[M + H]^+$. $C_{17}H_{17}O_2P$ (284.1): C 71.80, H 6.00, O 11.26; found C 71.57, H 6.03, O 11.58

Compound 6b: R_f = 0.32 (EtOAc); m.p. 62 °C (hexane/EtOAc). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.43-7.13 (m, 10 H), 6.9 (ddd, *J* = 51.0, *J* = 8.3, *J* = 2.8, 1 H), 6.2 (ddd, *J* = 22.8, *J* = 8.4, *J* = 2.3, 1 H), 5.16 (d, *J* = 9.4, 2 H), 4.11 (m, 1 H), 2.43 (ddd, *J* = 15.7, *J* = 13.6, *J* = 9.0, 1 H), 1.81 (ddd, *J* = 15.8, *J* = 13.8, *J* = 3.8, 1 H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 154.2 (d, *J* = 29.1), 142.4 (d, *J* = 4.4), 136.5 (d, *J* = 4.4), 129.0, 128.7, 128.5, 128.2, 127.3, 127.2, 124.1(d, *J* = 120.6), 66.9 (d, *J* = 5.8), 45.4 (d, *J* = 16.0), 30.7 (d, *J* = 94.5). ^{31}P NMR ($CDCl_3$, 121.5 MHz): δ = 74.5. MS (CI/NH_3): *m/z* (%) = 302 (37) $[M + NH_4]^+$, 285 (100) $[M + H]^+$. $C_{17}H_{17}O_2P$ (284.1): C 71.80, H 6.00, O 11.26; found C 71.87, H 6.13, O 11.21.

Methyl 5-(1-Benzyloxy-1-oxo-4-phenyl-3-phospholen-2-yl)pentanoate (7): LDA (1.5 mL, 0.85 M in tetrahydrofuran, 1.3 mmol) was added slowly at −78 °C to a solution of **6b** (340 mg, 1.2 mmol) and TMEDA (900 μL, 6 mmol) in dry tetrahydrofuran (5 mL). After 15 min, a solution of methyl 5-iodovaleroate (730 mg, 3.0 mmol) in dry tetrahydrofuran (5 mL) was added dropwise. After 1 h at −78 °C, the reaction mixture was hydrolyzed with a saturated aqueous solution of NH_4Cl (10 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic layers were washed with water (20 mL), dried with $MgSO_4$, filtered, and concentrated under vacuum. The crude residue was purified by silica gel chromatography (hexane/EtOAc, 3:2) to give **7** (145 mg, 35%) as a colorless oil; R_f = 0.40 (hexane/EtOAc, 3:2). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.39−7.29 (m, 10 H), 6.29 (m, 1 H), 5.14 (d, *J* = 9.0, 2 H), 3.67 (s, 3 H), 2.92−2.64 (m, 3 H), 2.34 (t, *J* = 7.4, 2 H), 1.95−1.48 (m, 6 H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 173.9, 136.7 (d, *J* = 17.4), 136.5 (d, *J* = 11.6), 136.3 (d, *J* = 5.8), 128.6, 128.5, 128.4, 128.2, 127.9, 126.4 (d, *J* = 14.5), 125.3, 66.3 (d, *J* = 7.3), 51.4, 41.5 (d, *J* = 88.6), 33.7, 30.7 (d, *J* = 90.1), 29.1, 27.7 (d, *J* = 7.3), 24.7; ^{31}P NMR ($CDCl_3$, 121.5 MHz): δ = 73.6. IR (neat): ν̃ = 2940, 2359, 1734, 1232, 1007 cm^{-1}. MS (CI/NH_3): *m/z* (%) = 416 (8) $[M + NH_4]^+$, 399 (100) $[M + H]^+$. $C_{23}H_{27}O_4P$ (398.2): C 69.37, H 6.83, O 16.07; found C 69.69, H 6.79, O 16.26.

Methyl 5-(1-Hydroxy-1-oxo-4-phenyl-3-phospholen-2-yl)pentanoate (8): TMSBr (215 mL, 1.7 mmol) was added slowly to a solution of compound **7** (265 mg, 0.66 mmol) in dichloromethane (2 mL). After 1 h at room temperature, methanol (0.5 mL) was added. After evaporation under vacuum, the product was purified by silica gel chromatography (CH_2Cl_2/MeOH, 4:1) to afford compound **8** (170 mg, 83%) as a white solid; R_f = 0.26 (CH_2Cl_2/MeOH, 4:1); m.p. > 280 °C (CH_2Cl_2/MeOH). ^{1}H NMR (CD_3OD, 300 MHz): δ = 7.42−7.19 (m, 5 H), 6.30 (d, *J* = 31.6), 3.64 (s, 3 H), 2.60 (d, *J* = 12.4), 2.50 (m, 1 H), 2.33 (t, *J* = 7.2, 3 H), 1.83−1.43(m, 6 H). ^{13}C NMR (CD_3OD, 50 MHz): δ = 176.1, 139.3 (d, *J* = 11.6), 138.5 (d, *J* = 16.0), 129.4, 128.8 (d, *J* = 13.1), 128.5, 126.3, 50.1, 44.6 (d, *J* = 91.5), 34.7, 34.3 (d, *J* = 90.1), 30.8, 29.3 (d, *J* = 8.7), 26.2. ^{31}P NMR (CD_3OD, 121.5 MHz): δ = 57.3. IR (KBr): ν̃ = 3412, 1736, 1181, 1053, 752 cm^{-1}.

5-(1-Hydroxy-1-oxo-4-phenyl-3-phospholen-2-yl)pentanoic Acid (1): Compound **8** (165 mg, 0.53 mmol) in dioxane (1.5 mL) was stirred overnight with LiOH (1 M, 2.7 mL, 2.7 mmol). After evaporation

under reduced pressure, the aqueous phase was acidified (pH = 1) with hydrochloric acid (4 M) and then extracted with chloroform (3 × 20 mL). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated under vacuum. The crude product was recrystallized from MeOH/CH_2Cl_2 (4:3) to give **1** (90 mg, 56%) as a white solid; m.p. > 280 °C (MeOH/CH_2Cl_2). ^{1}H NMR (CD_3OD, 300 MHz): δ = 7.45 (d, *J* = 6.8, 2 H), 7.37−7.28 (m, 3 H), 6.37 (m, 1 H), 2.83 (d, *J* = 12.8, 2 H), 2.74 (m, 1 H), 2.35 (t, *J* = 7.2, 2 H), 1.86−1.54 (m, 6 H). ^{13}C NMR (CD_3OD, 75 MHz): δ = 177.5, 138.2 (d, *J* = 13.1), 138.0 (d, *J* = 21.8), 129.6, 129.2, 127.8 (d, *J* = 13.5), 126.5, 43.8 (d, *J* = 90.1), 34.7, 32.6 (d, *J* = 91.6), 30.1 (d, *J* = 2.9), 28.9 (d, *J* = 7.3), 26.0. ^{31}P NMR (CD_3OD, 121.5 MHz): δ = 70.45. IR (KBr): ṽ = 3416, 2926, 2854, 1737, 1638, 1620, 1446, 1096, 798, 697, 670 cm^{-1}. HRMS calcd. for $C_{15}H_{19}O_4P$ 294.1021, found 294.1001.

Methyl 2-*tert*-Butoxycarbonylamino-3-phenylpropanoate (10): A solution of D,L-phenylalanine methyl ester hydrochloride (2.5 g, 11.6 mmol) in dry dichloromethane (25 mL) was treated with *tert*-butyldicarbonate (2.5 g, 11.6 mmol) in the presence of Et_3N (3.2 mL, 23.2 mmol). The mixture was stirred at room temperature for 4 h, then hydrolyzed with a saturated aqueous solution of NH_4Cl (50 mL). The aqueous phase was extracted with dichloromethane (2 × 50 mL). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated under vacuum. After silica gel chromatography (hexane/EtOAc, 9:1), product **10** (2.9 g, 90%) was obtained as a white solid; R_f = 0.27 (hexane/EtOAc, 9:1); m.p.102 °C (hexane/EtOAc). ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.35−7.17 (m, 5 H), 4.95 (m, 1 H), 4.61 (m, 1 H), 3.72 (s, 3 H), 3.11 (m, 2 H), 1.42 (s, 9 H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 172.3, 155.1, 135.9, 129.3, 128.5, 127.0 79.9, 54.4, 52.2, 38.3, 28.3. IR (KBr): ṽ = 3360, 2972, 1741, 1706, 1513, 1367, 1223, 1162 cm^{-1}. MS (CI/NH_3): *m*/*z* (%) =297 (89) $[M + NH_4]^+$, 280 (100) $[M + H]^+$.

***tert*-Butyl *N*-(2-Phenylallyl)-*N*-(1-phenylbut-3-en-2-yl)carbamate (9):** NaH (60%, 100 mg, 2 mmol) was added at 0 °C to a solution of compound **11** (325 mg, 1.3 mmol) and 3-bromo-2-phenylpropene (390 mg, 2 mmol) in dry dimethylformamide (6 mL). After 2 h, the ice bath was removed and the reaction mixture was stirred for 24 h at room temperature. The mixture was then treated with a saturated aqueous solution of NH_4Cl (6 mL) and the aqueous layer was extracted with diethyl ether (2 × 10 mL). The combined organic layers were washed with water (20 mL), and then dried with $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel chromatography (hexane/EtOAc, 98:2) to afford compound **9** (435 mg, 92%) as a colorless oil; R_f = 0.46 (hexane/EtOAc, 98:2). ^{1}H NMR ([D$_6$]DMSO, 300 MHz, 300 K, 2 rotamers δ = 7.42−7.19 (m, 10 H), 5.86 (m, 1 H), 5.37 (m, 1 H), 4.99 (m, 3 H), 4.46−4.15 (m, 2 H), 3.87 (m, 1 H), 2.90 (m, 2 H), 1.31 (s, 9 H). ^{1}H NMR ([D$_6$]DMSO, 300 MHz, 450 K) coalescence δ = 7.37−7.19 (m, 10 H), 5.94 (m, 1 H), 5.32 (s, 1 H), 5.07−4.97 (m, 3 H), 4.40 (m, 1 H), 4.17 (d, *J* = 16.1, 1 H), 3.97 (d, *J* = 16.1, 1 H), 2.98 (m, 2 H), 1.40 (s, 9 H). ^{13}C NMR ([D$_6$]DMSO, 75 MHz, 300 K): 2 rotamers, δ = 154.2, 144.3, 144.0, 138.6, 137.2, 129.2, 128.3, 128.1, 127.8, 126.1, 126.0, 116.3, 116.25, 113.2−112.5, 78.8, 60.3, 48.7 38.2−37.8, 27.9. ^{13}C NMR ([D$_6$]DMSO, 75 MHz, 325 K) coalescence δ = 154.1, 144.5, 138.4, 137.0, 129.0, 128.0, 127.9, 127.5−125.9, 116.1, 112.8, 78.7, 60.3, 48.8, 37.9, 27.8. IR (CsI): ṽ = 2977, 1695, 1169, 781, 700 cm^{-1}. HRMS calcd. for $C_{24}H_{29}NO$ ($M^{+\cdot}$) 263.2198, found 263.2190.

***tert*-Butyl 2-Benzyl-4-phenyl-2,5-dihydropyrrole-1-carboxylate (12):** A solution of compound **9** (440 mg, 1.1 mmol) in dry benzene (60 mL) was heated at 60 °C in the presence of **4** (16 mg, 0.019 mmol) for 6 h. After evaporation of the solvent, silica gel chromatography (hexane/EtOAc, 97:03) provided compound **12** (310 mg, 83%) as a yellow oil; R_f = 0.32 (hexane/EtOAc, 9:1). ^{1}H NMR ($CDCl_3$, 200 MHz): (2 rotamers ratio 3:2) *rotamer 1:* δ = 7.32−7.17 (m, 10 H), 6.00 (m, 1 H), 4.82 (m, 1 H), 4.58 (dt, *J* = 14.7, *J* = 1.7, 1 H), 4.23 (dt, *J* = 14.7, *J* = 1.7, 1 H), 3.29 (dd, *J* = 12.6, *J* = 3.3, 1 H), 2.80 (dd, *J* = 12.6, *J* = 8.8, 1 H), 1.60 (s, 9 H); *rotamer 2:* δ = 7.32−7.17 (m, 10 H), 6.05 (m, 1 H), 4.94 (m, 1 H), 4.43 (dt, *J* = 14.4, *J* = 1.6, 1 H), 4.23 (ddd, *J* = 14.5, *J* = 5.2, *J* = 2.0, 1 H), 3.33 (dd, *J* = 12.5, *J* = 3.3, 1 H), 2.94 (dd, *J* = 12.6, *J* = 8.4, 1 H), 1.60 (s, 9 H). ^{13}C NMR ($CDCl_3$, 75 MHz): (2 rotamers ratio 3:2) *rotamer 1:* δ = 154.2, 137.9, 133.2, 128.8, 128.1, 126.4, 125.4, 123.2, 79.5, 66.4, 53.6, 41.3, 28.7; *rotamer 2:* δ = 154.1, 137.3, 133.3, 128.8−128.1, 126.1, 125.5, 123.5, 79.9, 66.2, 53.9, 39.9, 27.9. IR (neat): ṽ = 2976, 1697, 1174, 752, 694 cm^{-1}. MS (CI/NH_3): *m*/*z* (%) = 353 (15) $[M + NH_4]^+$, 336 (100) $[M + H]^+$. $C_{22}H_{25}NO_2$ (335.4): C 78.77, H 30.28, O 9.54; found C 78.55, H 30.33, O 9.21.

2-Benzyl-4-phenyl-2,5-dihydro-1*H*-pyrrole (13): Compound **12** (235 mg, 0.69 mmol) was dissolved in a 20% solution of TFA in dichloromethane (10 mL). After 30 min at room temperature, the solvents were evaporated under reduced pressure and the residue purified by silica gel chromatography (CH_2Cl_2/MeOH, 95:05) to give compound **13** (185 mg, 98%) as a colorless oil; R_f = 0.31 (CH_2Cl_2/MeOH, 9:1). ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.37−7.20 (m, 10 H), 6.13 (m, 1 H), 4.51 (m, 1 H), 4.22 (ddd, *J* = 13.9, *J* = 4.0, *J* = 1.7, 1 H), 4.11 (ddd, *J* = 13.9, *J* = 3.0, *J* = 2.2, 1 H), 3.35 (dd, *J* = 13.5, *J* = 5.8, 1 H), 3.10 (dd, *J* = 13.6, *J* = 9.1, 1 H), 1.99 (br. s, 1 H). IR (neat): ṽ = 3364, 3060, 3027, 2917, 2844, 1694, 1682, 1600, 1495, 1454, 1404, 1337, 1200, 1130, 1076, 757, 692. cm^{-1}. MS (CI/NH_3): *m*/*z* (%) = 236 (100) $[M + H]^+$.

Methyl 5-(2-Benzyl-4-phenyl-2,5-dihydropyrrol-1-yl)pentanoate (14): Methyl 5-bromovaleroate (230 mL, 1.6 mmol) and K_2CO_3 (1.4 g, 2 mmol) were added to a solution of compound **13** (300 mg, 1.3 mmol) in acetonitrile (10 mL). After 3 h under reflux, the mixture was filtered and the filtrate was concentrated under reduced pressure. Silica gel chromatography (CH_2Cl_2/MeOH, 95:5) provided compound **14** (380 mg, 85%) as a yellow oil; R_f = 0.29 (CH_2Cl_2/MeOH, 95:5). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.36−7.23 (m, 10 H), 6.02 (m, 1 H), 4.26 (ddd, *J* = 12.8, *J* = 4.5, *J* = 1.1, 1 H), 3.78 (m, 1), 3.57 (ddd, *J* = 12.9, *J* = 4.9, *J* = 2.2, 1 H), 3.00 (dd, *J* = 13.2, *J* = 5.3, 1 H), 2.81 (dt, *J* = 12.0, *J* = 7.9, 1 H), 2.72 (dd, *J* = 13.2, *J* = 9.1, 1 H), 2.55 (d, *J* = 12.1, *J* = 6.4, 1 H), 2.37 (t, *J* = 7.4, 2 H), 1.81−1.55 (m, 4 H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 174.1, 139.4, 138.6, 134.3, 129.4, 128.4, 128.45, 127.6, 126.1, 125.5, 125.1, 73.8, 60.6, 54.6, 51.5, 42.5, 33.9, 28.6, 22.8. IR (neat): ṽ = 3056, 3023, 2933, 2866, 2777, 1733, 1594, 1488, 1444, 1338, 1244, 1194, 1166, 1122, 1077, 749, 694 cm^{-1}. MS (CI/NH_3): *m*/*z* (%) = 350 (100) $[M + H]^+$. HRMS calcd. for $C_{23}H_{28}NO_2$ ($M^{+\cdot}$) 350.2120, found 350.2123

2-Benzyl-1-(4-carboxybutyl)-1-methyl-4-phenyl-2,5-dihydro-1*H*-pyrrolium Iodide (3): Compound **14** (100 mg, 0.29 mmol) in dioxane (1 mL) was treated with LiOH (1 M, 1.4 mL, 1.4 mmol). After having been stirred overnight at room temperature, the mixture was acidified with dilute hydrochloric acid (2 mL). The aqueous layer was extracted with chloroform (3 × 10 mL) and the resulting organic layers were dried with $MgSO_4$, filtered, and concentrated under vacuum. The acid (50 mg) was obtained and, without further purification, treated with MeI in acetone (30 μL, 0.48 mmol). After the mixture had been stirred overnight at room temperature, diethyl ether was added and the precipitated compound **3** was isolated as a yellow gum (130 mg, quantitative yield). ^{1}H NMR ($CDCl_3$, 300 MHz): 2 diastereomers, 1:1, δ = 7.41−7.23 (m, 10 H), 6.90

(br. s, 1 H), 5.87−5.81 (m, 1 H), 5.17−4.68 (m, 3 H), 4.22−2.89 (m, 4 H), 3.48 and 3.39 (s and s, 3 H), 2.54−2.37 (m, 2 H), 1.85−1.66 (m, 4 H). ^{13}C NMR ($CDCl_3$, 75 MHz): 2 diastereomers, 1:1, δ = 177.1 and 176.9, 136.8 and 136.2, 134.6 and 134.4, 130.7−126.3, 121.4 and 119.8; 82.3 and 81.1, 69.9 and 68.6, 64.5 and 59.4, 51.7 and 46.3, 36.4 and 35.0, 30.9, 23.9, 23.7, 22.3 and 22.0. IR (neat): $\tilde{\nu}$ = 2938, 1723, 1455, 1189, 755 cm^{-1}. HRMS calcd. for $C_{23}H_{28}NO_2$ ($M^{+\cdot}$) 350.2120, found 350.2115.

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[1] [1a] K. D. Janda, C. G. Shevlin, R. A. Lerner, *Science* **1993**, *259*, 490−493. [1b] V. E. Gouverneur, K. N. Houk, B. de Pascual-Teresa, B. Beno, K. D. Janda, R. A. Lerner, *Science* **1993**, *262*, 204−208. [1c] J. Na, K. N. Houk, C. G. Shevlin, K. D. Janda, R. A. Lerner, *J. Am. Chem. Soc.* **1993**, *115*, 8453−8454. [1d] B. F. Cravatt, J. A. Ashley, K. D. Janda, D. L. Boger, R. A. Lerner, *J. Am. Chem. Soc.* **1994**, *116*, 6013−6014. [1e] K. Gruber, B. Zhou, K. N. Houk, R. A. Lerner, C. G. Shevlin, I. A. Wilson, *Biochemistry* **1999**, *38*, 7062−7074.

[2] For selected examples: [2a] J. C. Ruble, J. Tweddell, G. C. Fu, *J. Org. Chem.* **1998**, *63*, 2794−2795. [2b] O. Fujimura, R. H. Grubbs, *J. Am. Chem. Soc.* **1996**, *118*, 2499−2500. [2c] M. Tokunaga, J. F. Larrow, F. Kakiuchi, E. N. Jacobsen, *Science* **1997**, *277*, 936−938. [2d] R. Chevenert, M. Letourneau, *Chem. Lett.* **1986**, 1151−1154. [2e] M. Beller, M. Eckert, H. Geissler, B. Napiersky, H.-P. Rebenstcok, E. W. Holla, *Chem. Eur. J.* **1998**, *4*, 935−941. [2f] J. M. Turner, T. Bui, R. A. Lerner, C. F. Barbas III, B. List, *Chem. Eur. J.* **2000**, *6*, 2772−2774.

[3] For reviews: [3a] H. Stecher, K. Faber, *Synthesis* **1997**, 1−17. [3b] R. S. Ward, *Tetrahedron: Asymmetry* **1995**, *6*, 1475−1490.

[4] [4a] S. Takahashi, *Prog. Ind. Microbiol.* **1986**, *24*, 269−279. [4b] C. J. Sih, R.-L. Gu, J. Z. Crich, R. Brieva, in: *Stereocontrolled Organic Synthesis*, (Ed.: B. M. Trost), Blackwell, Oxford, **1994**, pp. 401−404. [4c] R. Noyori, *Science* **1990**, *248*, 1194−1199. [4d] R. Noyori, H. Takaya, *Acc. Chem. Res.* **1990**, *23*, 345−350. [4e] A. Kubo, H. Kubota, M. Takahashi, K. Nunami, *J. Org. Chem.* **1997**, *62*, 5830−5837. [4f] R. Stürmer, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1173−1174. [4g] A. L. E. Larsson, B. A. Persson, J. E. Backvall, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1211−1212. [4h] B. A. Persson, A. L. E. Larsson, M. Le Ray, J. E. Backvall, *J. Am. Chem. Soc.* **1999**, *121*, 1645−1650. [4i] B. A. Persson, F. F. Huerta, J. E. Backvall, *J. Org. Chem.* **1999**, *64*, 5237−5240. [4j] F. F. Huerta, Y. R. S. Laxmi, J. E. Backvall, *Org. Lett.* **2000**, *2*, 1037−1040. [4k] O. Pamies, J. E. Backvall, *J. Org. Chem.* **2001**, *66*, 4022−4025.

[5] [5a] H. S. Bevinakatti, R. V. Newadkar, A. A. Banerji, *J. Chem. Soc., Chem. Commun.* **1990**, 1091−1092. [5b] H. S. Bevinakatti, A. A. Banerji, R. V. Newadkar, A. A. Mokashi, *Tetrahedron: Asymmetry* **1992**, *3*, 1505−1508.

[6] [6a] R.-L. Gu, I.-S. Lee, C. J. Sih, *Tetrahedron Lett.* **1992**, *33*, 1953−1956. [6b] J. Z. Crich, R. Brieva, P. Marquart, S. Flemming, C. J. Sih, *J. Org. Chem.* **1993**, *58*, 3252−3258.

[7] For recent results on chemical and enzymatic DKR with oxazolones: [7a] L. Xie, W. Hua, A. S. C. Chan, Y.-C. Leung, *Tetrahedron: Asymmetry* **1999**, *10*, 4715−4728. [7b] N. J. Turner, J. R. Winterman, R. McCague, J. S. Parratt, S. J. C. Taylor, *Tetrahedron Lett.* **1995**, *36*, 1113−1116. [7c] S. A. Brown, M. C. Parker, N. J. Turner, *Tetrahedron: Asymmetry* **2000**, *11*, 1687−1690.

[8] For examples of antibody-promoted ester hydrolysis: [8a] A. Tramontano, K. D. Janda, R. A. Lerner, *Science* **1986**, *234*, 1566−1570. [8b] S. J. Pollack, J. W. Jacobs, P. G. Schultz, *Science* **1986**, *234*, 1570−1573. [8c] J. D. Stevenson, N. R. Thomas, *Nat. Prod. Rep.* **2000**, 535−577.

[9] [9a] A. Williams, *J. Chem. Soc., Perkin Trans. 2* **1975**, 947−953. [9b] J. de Jersey, P. Willadsen, B. Zerner, *Biochemistry* **1966**, *8*, 1959−1966. [9c] J. de Jersey, A. A. Kortt, B. Zerner, *Biochem. Biophys. Res. Commun.* **1966**, *23*, 745−750. [9d] M. S. Matta, M. E. Andracki, *J. Am. Chem. Soc.* **1985**, *107*, 6036−6039.

[10] For reviews on hapten design for the generation of catalytic antibodies: [10a] E. Keinan, R. A. Lerner, *Isr. J. Chem.* **1996**, *36*, 113−119. [10b] N. Thomas, *Nat. Prod. Rep.* **1996**, *13*, 479−511. [10c] N. Thomas, *Appl. Biochem. Biotechnol.* **1994**, *47*, 345−372. [10d] M. M. Mader, P. A. Bartlett, *Chem. Rev.* **1997**, *97*, 1281−1301. [10e] J. D. Stevenson, N. R. Thomas, *Nat. Prod. Rep.* **2000**, 535−577.

[11] F. Tanaka, K. Kinoshita, R. Tanimura, I. Fujii, *J. Am. Chem. Soc.* **1996**, *118*, 2332−2339.

[12] About the importance of the anionic character mimicry: H. Kakinuma, K. Shimazaki, N. Takahashi, K. Takahashi, S. Niihata, Y. Aoki, K. Hamada, H. Matsushita, Y. Nishi, *Tetrahedron* **1999**, 2559−2572.

[13] About "bait and switch" strategy: [13a] R. A. Lerner, S. J. Benkovic, *Bioassays* **1988**, *9*, 107. [13b] K. D. Janda, *Biotechnol. Prog.* **1990**, 6, 178−181. [13c] K. D. Janda, M. I. Weinhouse, D. M. Schloeder, R. A. Lerner, *J. Am. Chem. Soc.* **1990**, *112*, 1274−1275. [13d] K. D. Janda, M. I. Weinhouse, T. Danon, K. A. Pacelli, D. M. Schloeder, *J. Am. Chem. Soc.* **1991**, *113*, 5427−5434. [13e] J. D. Stevenson, N. R. Thomas, *Nat. Prod. Rep.* **2000**, 535−577.

[14] For reviews on RCM: [14a] S. Blechert, M. Schuster, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2037−2056. [14b] H. G. Schmalz, *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1833−1836. [14c] R. H. Grubbs, S. Chang, *Tetrahedron* **1998**, *54*, 4413−4450. [14d] S. K. Armstrong, *J. Chem. Soc., Perkin Trans. 1* **1998**, 371−388.

[15] [15a] P. Schwab, M. B. France, J. W. Ziller, R. H. Grubbs, *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2039−2041. [15b] P. Schwab, R. H. Grubbs, J. W. Ziller, *J. Am. Chem. Soc.* **1996**, *118*, 100−110.

[16] R. R. Schrock, J. S. Murdzek, G. C. Bazan, J. Robbins, M. Dimare, M. O'Regan, *J. Am. Chem. Soc.* **1990**, *112*, 3875−3886.

[17] M. Bujard, V. Gouverneur, C. Mioskowski, *J. Org. Chem.* **1999**, *64*, 2119−2123.

[18] For references on the use of aryldiazonium salts in palladium-catalyzed Heck reactions: [18a] G. Mehta, S. Sengupta, *Tetrahedron Lett.* **1996**, *37*, 8625−8626. [18b] S. Sengupta, S. Batthacharyya, *Synth. Commun.* **1996**, *26*, 231−236. [18c] S. Darses, T. Jeffery, J. P. Genet, J. L. Brayer, J. P. Demoute, *Tetrahedron Lett.* **1996**, *37*, 3857−3860. [18d] S. Darses, J. P. Genet, J. L. Brayer, J. P. Demoute, *Tetrahedron Lett.* **1997**, *38*, 4393−4396. [18e] S. Sengupta, S. Batthacharyya, S. K. Sadhukhan, *J. Chem. Soc., Perkin Trans. 1* **1998**, 275−277. [18f] S. Sengupta, S. K. Sadhukhan, S. Batthacharyya, J. J. Guha, *J. Chem. Soc., Perkin Trans. 1* **1998**, 275−277.

[19] For palladium-catalyzed reactions of 1-phenylphospholene-1-oxide with aryl iodide and aryldiazonium salts, see: P. Desmazeau, J.-Y. Legros, J.-C. Fiaud, *Tetrahedron Lett.* **1998**, *39*, 6707−6710.

[20] C. E. McKenna, M. T. Higa, N. H. Cheung, M.-C. McKenna, *Tetrahedron Lett.* **1977**, *2*, 155−158.

[21] M. Bujard, A. Briot, V. Gouverneur, C. Mioskowski, *Tetrahedron Lett.* **1999**, *41*, 8785−8788.

[22] [22a] Z.-Y. Wei, E. Knaus, *Synthesis* **1994**, 1463−1466. [22b] S. Kobayashi, M. Isobe, M. Ohno, *Tetrahedron Lett.* **1984**, *25*, 5079−5082.

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