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Preparation of heterocyclic compounds via carbon–carbon bond formation catalyzed by an antibody

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Abstract

A monoclonal antibody, elicited by a transition-state analogue, acted as an enzymelike catalyst for the formation of carbon–carbon bond. The generation of carboanion by the action of abzyme and the internal nucleophilic attack on the activated functional group, such as the carbonyl and/or the imine group with a fluoroalkyl group, producing the corresponding δ-lactones, are described.

Keywords: Abzyme; Antibody; Carbon–carbon bond formation

1. Introduction

Recent developments in the design of transition state (TS)-analogue concept are having an important impact on a various types of organic reactions. [1–5]. Particularly, the TS-analogues analogues of the disfavored chemical transformation, [6,7] enantioselective protonation, [8] nucleophilic substitution reaction, [9] peptide synthesis, [10] syn elimination, [11] and SP^3 carbon-type in the hydrolysis of carbonate [12] have attracted the interest of synthetic chemists. A fundamental objective of the hapten design is to select the TS-analogues with an environment complementary in structure and electric distribution to a TS of a given reaction. In fact, we have previously reported that α,α-difluoroketones, which have a highly electrophilic carbonyl group, exist in the stable hydrate from in aqueous media and as a result serve as ideal structural mimics of the putative tetrahedral intermediates. [12] However, in spite of the above mentioned pioneering TS-analogue designs, studies on the TS-analogue design and synthesis of antibody catalysts for the carbon–carbon bond formation remain an unsolved problem. To date, carbon–carbon bond formation is a reaction that has been used infrequently in synthesis, particularly from the view point of stereocontrol. Furthermore, the challenge of preparing a new type of TS-analogue, for the development of antibody reagents with higher reaction selectivities remains.

Accordingly, we have devoted our attention to the development of antibody catalysts for the preparation of a variety of functionalized heterocycles via carbon–carbon bond formation.

2. Results and discussion

A fundamental concept of antibody catalysts design for the carbon–carbon bond formation is

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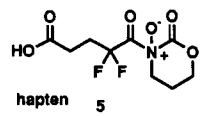
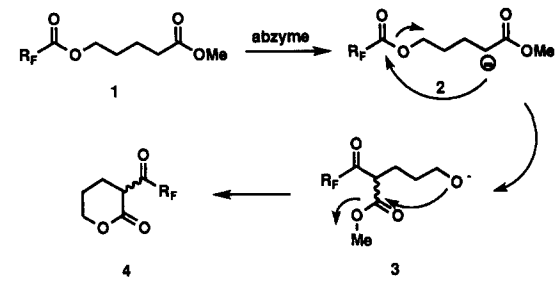
to select haptens with a generation source of activated carbanion and an activated functional group attacked by the generated carbanion.

We anticipated that antibodies specific for hapten **5**



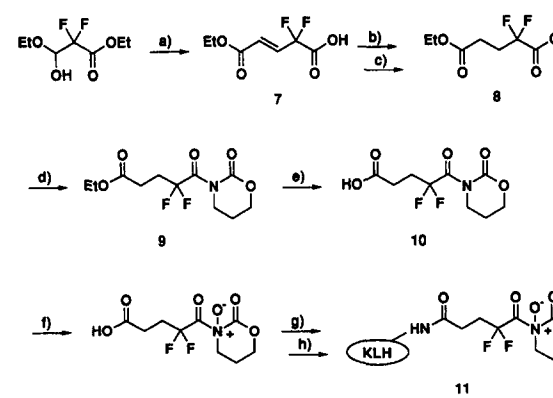
might catalyze the carbon–carbon bond forming reaction and cyclization on the basis of the following considerations: (1) Nucleophilic addition to a carbonyl necessitates the addition of electrons from the HOMO of the nucleophile to the LUMO (the acceptor orbital) of the carbonyl. The acceptor energy is the initial energy of the LUMO level prior to perturbation due to the incoming nucleophile and can therefore provide insight into the energetics of the initial stages of the reaction. the amount of stabilization (ΔE_s) for the HOMO–LUMO interaction is inversely proportional to the initial energy difference of the HOMO and LUMO (ΔE) such that decreasing the LUMO energy or increasing the HOMO energy would serve to decrease ΔE and increase ΔE_s. Stabilization of the acceptor level can be caused by an inductive electron withdrawing effect. Based on the PM3 calculations of the corresponding methyl acetate, [13] these results show the acceptor level energy (LUMO) of the fluoroesters ($CHF_2CO_2CH_3$; 0.2135 eV, $CF_3CO_2CH_3$; −0.1479 eV) as consistently lower than that for the analogous methyl ester ($CH_3CO_2CH_3$; 1.02127 eV). (2) The cyclization to produce δ-lactone should ensue from the negatively-charged alkoxy moiety, which is generated by attack on the carbonyl group by the carbanion. (3) The prochiral methylene proton should discriminate with the chiral environment provided by the antibody combining site (Scheme 1).

The antigen required was prepared as shown in Scheme 2. To form the desired antibody reagents, an immunogenic conjugate was pre-



Scheme 1.

pared by reaction of the compound **11** with a carrier protein (keyhole limpet hemocyanin or bovine serum albumin). Lymphocytes from the soleen of BALB/c mice immunized with each type of the purified antigens (the KLH–hapten conjugate or the BSA–hapten conjugate) were fused by standard protocols using mouse myeloma cell (P3/NS1/1-Ag4-1) as the fusion partner. Antibodies were screened by ELISA for cross-reactivity with the KLH–hapten conjugate or the BSA–hapten conjugate, ie., for the inhibitor of binding to the KLH–hapten conjugate by free hapten. Five or nine antibodies were obtained for each hapten. Antibodies were purified from ascites fluid by protein A Sepharose 4B affinity chromatography and were determined to be > 95% homogeneous by sodium dodecyl sulfate polyacrylamide gel electrophoresis.



Scheme 2. (a) $EtO_2CCH_2P(O)(OEt)_2$, NaH, THF. (b) H_2, 10% Pd–C, EtOH. (c) $C_6H_4(COCl)_2$, reflux. (d) n-BuLi, THF, 2,3,5,6-tetrahydro-4H-1,3-oxazine-2-one. (e) lipase–MY, H_2O. (f) mCPBA, CH_2Cl_2. (g) Keyhole limpet hemocyanin (KLH), 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride, phosphate buffer pH 6.0. (h) Dialysis, NaCl buffer, pH 7.4.

Kinetic constants were determined by the method of initial rates data. Kinetic parameters for the reaction from the Lineweaver–Burk plots were determined. The value K_{cat} and Michaelis constant K_m, were found to be 0.89 ± 0.2 min^{-1} and 440 ± 70 μM, respectively.

This antibody-catalyzed reaction was inhibited by the addition of hapten. The inhibition constant for the ($K_i = 25 \pm 4$ μM at 25°C) for the formation of the antibody-compound **11** complex was determined by measuring the rate of cyclization in the presence of antibody (30 μM) at vary inhibitor concentrations. Obviously, these reactions did not proceed in the absence of obtained antibody.

The antibody acted to promote the carbon–carbon bond-forming reaction and cyclization to produce 3-difluoroacetyl tetrahydro-2-pyrone (**15**; 64% yield; $[\alpha]_D^{24}$ +14.1 (*c* 1.04, $CHCl_3$)) with selectivity of > 56% ee or 3-trifluoroacetyl tetrahydro-2-pyrone (**16**; 57% yield; $[\alpha]_D^{24}$ +13.4 (*c* 1.13, $CHCl_3$)) with selectivity of > 54% ee.

3. Experimental

3.1. General procedures

All commercially available reagents were used without further purification. Infrared spectra were obtained by using a JASCO A-102 or a Jasco FT/IR-5000 spectrometer and KBr pellets. Nuclear magnetic resonance (NMR) spectra were recorded at 200 MHz or 500 MHz for ^{1}H NMR (internal Me_4Si) and at 470 MHz for ^{19}F NMR (internal C_6F_6) at 125 MHz for ^{13}C NMR in $CDCl_3$. Yields were those of isolated products.

3.2. Synthesis of antigen *11*

trans-Ethyl 4-carboxy-4,4-difluoro-2-butenoate **7**. To a suspension of sodium hydride (0.65 g, 2.5 mmol) in THF (5 ml) was added triethyl phosphonoacetate (0.49 g, 2.2 mmol) at 0°C, and then the mixture was stirred at ambient temperature for 10 min. Ethyl 3-ethoxy-2,2-difluoro-3-hydroxypropionate [11] (0.40 g, 2.0 mmol) was then added dropwise, and the whole was stirred at ambient temperature for 2 h. After dilution with diethyl ether (15 ml), the mixture was poured into 1 N HCl (20 ml). The organic layer was separated and dried over magnesium sulfate. On removal of the solvent, flash chromatography on silica gel with ethyl acetate yield half ester **7** in 54%: ^{1}H NMR: δ 1.34 (3 H, t, $J_{H,H} = 7.1$ Hz), 4.31 (2 H, q, $J_{H,H} = 7.1$ Hz), 6.43 (1 H, dt, $J_{H,H} = 2.4$, 15.1 Hz), 6.87 (1 H, dt, $J_{H,H} = 11.0$, 15.1 Hz), 10.6 (1 H); ^{13}C NMR; δ 14.8, 63.5, 111.7 (CF_2, t, $J = 250$ Hz), 128.1 (t, $J = 8.5$ Hz), 135.0 (t, $J = 25.1$ Hz), 162.0 (t, $J = 32.7$ Hz), 164.7; ^{19}F NMR: δ 56.8 (dd, $J = 3.2$, 11.0 Hz); IR: 1730, 1710 cm^{-1}.

3.3. Compound *8*

(a) To a suspension of Pd–C (10%, 0.1 g) in ethanol (50 ml), a solution of compound **7** (1.94 g, 10 mmol) in ethanol (10 ml) was added under a hydrogen atmosphere at room temperature. After 24 h of stirring, the catalyst was removed by filtration. On removal of the solvent, the title material **8** was obtained by column chromatography on silica gel using by ethyl acetate in > 85% yield: ^{1}H NMR: δ 1.56 (3H, t, $J_{H,H} = 7.0$ Hz), 2.27–2.46 (2 H, m), 2.51–2.57 (2 H, m), 4.24 (2 H, q, $J_{H,H} = 7.0$ Hz), 10.5 (1 H); ^{13}C NMR; δ 14.2, 27.7, 28.8, 63.9, 116.5 (CF_2, t, $J = 251$ Hz), 161.2, 167.4; ^{19}F NMR: δ 57.4 (dd, $J = 3.4$, 11.5 Hz); IR:1725, 1710 cm^{-1}.

(b) A solution of obtained half acid (10 mmol) and phthaloyl dichloride (2.03 g, 10 mmol) was heated at 100°C for 3 h, and then compound **8** was distilled under dynamic vacuum.

3.4. Compound *9*

To a solution of 2,4,5,6-tetrahydro-4H-1,3-oxazine-2-one (1.01 g, 10 mmol) in THF (30 ml) was added *n*-butyllithium in hexane (2.5 M, 4.0 ml, 10 mmol) at −78°C. After 30 min of

stirring at that temperature, compound **8** (8 mmol) was added to the solution. The whole solution was stirred at −78°C for 1 h and then allowed to warm to room temperature. After 1 h of stirring, the mixture was quenched with 1 N HCl and then extracted with diethyl ether. The extract was dried over anhydrous $MgSO_4$ and the solvent removed. Flash chromatography (silica gel, 5:1 hexane–EtOAc) afforded compound **9** in 59% yield: ^{1}H NMR: δ 1.49 (3 H, t, $J_{H,H}$ = 7.2 Hz), 2.24–2.59 (6 H, m), 3.47–3.68 (4 H, m), 4.24 (2 H, q, $J_{H,H}$ = 7.0 Hz); ^{13}C NMR; δ 14.4 27.1, 28.3, 30.2, 62.3, 64.1, 65.3, 115.9 (CF_2, t, J = 254 Hz), 162.5, 164.3, 167.1; ^{19}F NMR: δ 58.4 (dd, J = 2.7, 12.1 Hz); IR: 1730, 1710, 1685 cm^{-1}. Analysis: Calcd for $C_{11}H_{15}NO_5F_2$: C, 47.32; H, 5.41; N, 5.02%. Found: C, 47.04; H, 5.65; N, 4.87%.

3.5. Compound 10

A suspension of compound **9** (2.79 g, 10 mmol) and lipase–MY (5 g, Meito Sangyo Co. Ltd.) in H_2O (100 ml) was stirred at 40°C for 24 h, and then the reaction was quenched with 1 N HCl. The corresponding acid was extracted with ethyl acetate. On removal of the solvent, flash chromatography on silica gel was afforded compound **10** in 75% yield: ^{1}H NMR: δ 2.21–2.64 (6 H, m), 3.39–3.65 (4 H, m), 10.7 (1 H). ^{13}C NMR; δ 27.1, 28.3, 30.2, 64.1, 65.3, 114.7 (CF_2, t, J = 251 Hz), 161.8, 164.1, 167.5; ^{19}F NMR: δ 58.7(dd, J = 2.5, 11.9 Hz); IR:1730, 1710, 1690 cm^{-1}. Analysis: Calcd for $C_9H_{11}NO_5F_2$: C, 43.04; H, 4.41; N, 5.58%. Found: C, 43.39; H, 4.38; N, 5.81%.

3.6. Antigen 11

A solution of compound **10** (5 mmol) and mCPBA (6 mmol) in methylene chloride (20 ml) was stirred at room temperature for 3 h, and then the solvent was removed under a dynamic vacuum. Into a mixture of obtained compound (40 mg, 0.15 mmol) in distilled water (4.5 ml), KLH (50 mg) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodimide hydrochloride (57 mg, 0.3 mmol) in phosphate buffer (pH 6.0, 15 ml) was added. After 24 h of stirring at room temperature, the precipitate was washed with 10% aq. $NaHCO_3$ and 1 N HCl, and then the dialysis was carried out in NaCl buffer (pH 7.4). The product was purified by chromatography on Sephadex G-50.

Synthesis of 3-difluoroacetyl tetrahydro-2-pyrone with antibody. A mixture of the antibody (30 μM, Lowry assay, with a molecular weight of 1.5×10^5 for immunoglobulin G) was incubated at 25°C in 50 ml of phosphate buffer (pH 7.3) and compound **13** (1.05 g, 5 mM) in acetonitrile (5 ml) was stirred at 25–27°C. After being stirred for 15 h, oily materials were extracted with diethyl ether. The ethereal extract was dried over anhydrous magnesium sulfate and the solvent removed. Flash chromatography on silica gel (*n*-hexane-ethyl acetate; 5:1) afforded the title material in 64% yield; $[\alpha]_D^{24}$ +14.1 (*c* 1.04, $CHCl_3$), > 56% ee; ^{1}H NMR: δ 1.57–1.83 (4 H, m), 3.79–3.91 (1 H, dd, $J_{H,H}$ = 3.4, 5.7 Hz), 4.15–4.27 (2 H, m), 5.57 (1 H, t, $J_{H,F}$ = 54.1 Hz); ^{13}C NMR; δ 27.5, 28.4, 31.4, 63.7, 114.0 (CF_2, t, J = 254 Hz), 162.4, 165.9; ^{19}F NMR: δ 27.8 (d, J = 54.8 Hz); IR:1780, 1715 cm^{-1}. Analysis: Calcd for $C_7H_8O_3F_2$: C, 47.20; H, 4.53%. Found: C, 47.53; H, 4.74%.

Synthesis of 3-trifluoroacetyl tetrahydro-2-pyrone with antibody. A mixture of the antibody (30 μM, Lowry assay, with a molecular weight of 1.5×10^5 for immunoglobulin G) was incubated at 25°C in 50 ml of phosphate buffer (pH 7.3) and compound **14** (1.14 g, 5 mM) in acetonitrile (5 ml) was stirred at 25–27°C. After being stirred for 15 h, oily materials were extracted with diethyl ether and worked up similarly. The title material was obtained in 57% yield; $[\alpha]_D^{24}$ +13.4 (*c* 1.13, $CHCl_3$), > 54% ee; ^{1}H NMR: δ 1.54–1.86 (4 H, m), 3.75–3.87 (1 H, dd, $J_{H,H}$ = 3.1, 5.5 Hz), 4.10–4.26 (2 H, m); ^{13}C NMR; δ 27.1, 28.5, 30.9, 62.8, 116.0 (q, J = 289 Hz), 166.8, 174.9; ^{19}F NMR: δ 81.5; IR: 1785, 1720 cm^{-1}. Analysis: Calcd for

$C_7H_7O_3F_3$: C, 42.87; H, 3.60%. Found: C, 43.09; H, 3.47%.

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