Antibody-catalyzed formation of a 14-membered ring lactone

Michael D. Pungente, Larry Weiler, and Hermann J. Ziltener

Abstract: Monoclonal antibody (MAb) F123, raised against a macrocyclic phosphonate transition-state analogue, catalyzed an intramolecular transesterification of the corresponding hydroxy ester to give a 14-membered ring lactone. The MAb reaction displayed enzyme-like Michaelis–Menten kinetics with a K_m of 255 μM and a k_{cat} of 0.01 min^{-1} based on *p*-nitrophenol release and calculated on an active-site basis. Substrate specificity and competitive inhibition by a transition state analogue (K_i = 3 μM) demonstrated that the catalytic activity was associated with binding in the antibody-combining site. The lactone product was isolated from a large-scale catalytic experiment through ether extraction and identified by gas chromatography – mass spectroscopy.

Key words: macrocyclization, lactones, catalytic antibodies.

Résumé : L'anticorps monoclonal (AcM) F123, élevé contre un analogue d'état de transition phosphonate macrocyclique, catalyse une réaction de transestérification intramoléculaire de l'ester hydroxylé correspondant pour conduire à la formation d'un cycle lactonique à quatorze chaînons. La réaction de l'AcM présente une cinétique de Michaelis–Menten semblable à celle observée avec les enzymes avec un K_m de 255 μM et un k_{cat} de 0,01 min^{-1} basé sur l'élimination du *p*-nitrophénol et calculé sur une base de site actif. La spécificité du substrat et l'inhibition compétitive par un analogue de l'état de transition (K_i = 3 μM) démontrent que l'activité catalytique est associée à la fixation dans un site de combinaison d'anticorps. La lactone produite lors d'une expérience catalytique à grande échelle a été isolée par extraction à l'éther et on l'a identifiée par spectrométrie de masse liée à un chromatographe en phase gazeuse.

Mots clés : macrocyclisation, lactones, anticorps catalytiques.

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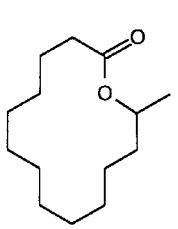
Introduction

The macrolide antibiotics contain a ring moiety, in many cases represented by a 14-membered lactone. The syntheses of these large-ring compounds pose a challenge for chemists, particularly in controlling the ring-forming step. Attempts to use enzymes in aqueous and organic solvents to catalyze medium to large ring lactonizations have met with limited success, particularly with secondary alcohols (1). Antibodies raised against transition state analogues have offered an alternative class of catalysts for complex reactions, many of which have been highlighted in review articles (2). Indeed, in 1987 Napper et al. (3) reported that an antibody raised against a transition state analogue for an intramolecular six-membered ring cyclization acted as a catalyst for the appropriate substrate to give a δ-lactone. To our knowledge, no antibody-catalyzed macrocyclization has been reported to date. To this end, we extended this antibody methodology to the formation of the 14-membered ring lactone **1**. We therefore synthesized the 14-membered ring transition state analogue **2**, which we subsequently conjugated to a carrier protein for the production of monoclonal antibodies.[4]



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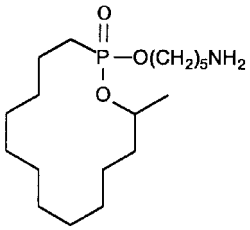

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M. Pungente[1,2] and L. Weiler.[3] Department of Chemistry, The University of British Columbia, Vancouver, BC V6T 1Z1, Canada. **H. Ziltener.** The Biomedical Research Centre and Department of Pathology and Laboratory Medicine, The University of British Columbia, Vancouver, BC V6T 1Z1, Canada.

[1]Present address: Department of Chemistry, Lakehead University, 955 Oliver Road, Thunder Bay, ON P7B 5E1, Canada.
[2]Corresponding author (e-mail: mike.pungente@lakeheadu.ca).
[3]Deceased 28 April, 1999.
[4]Supplementary data (Methods) may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0S2, Canada (http://www.nrc.ca/cisti/irm/unpub_e.shtml for information on ordering electronically).

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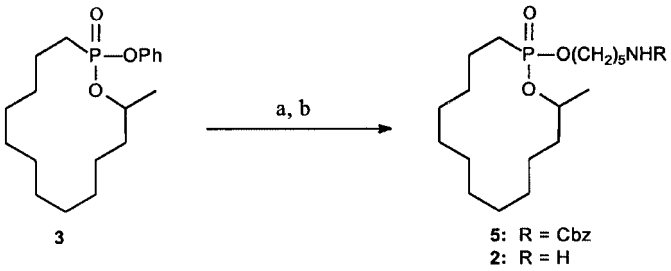
Scheme 1. (*a*) *n*-BuLi, THF, –78°C, $HO(CH_2)_5NHCbz$ (**4**), 59%. (*b*) H_2, 10% Pd/C, EtOH, 85%.



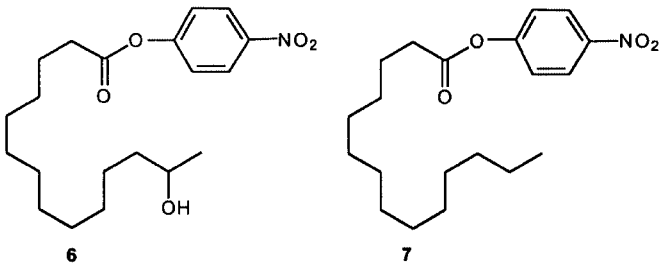
Results and discussion

The synthesis of hapten **2** (Scheme 1) was achieved by substitution of the phenyl moiety of the S*R* diastereomer of phosphonate **3** (4) with the anion of alcohol **4** to give **5** in a 59% yield.

Hydrogenolysis of **5** in ethanol with hydrogen and 10% Pd/C afforded amine **2** in 85% yield.

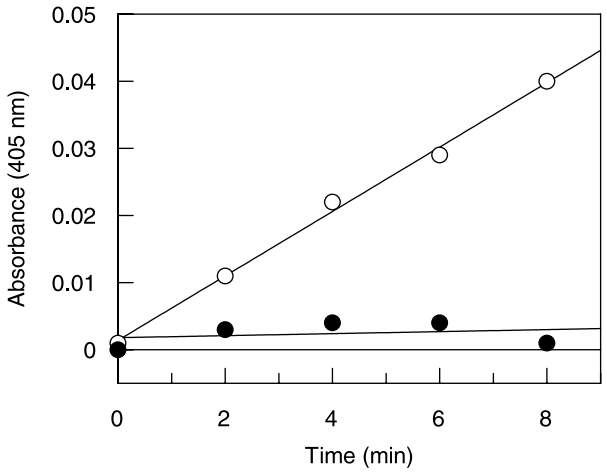
Amine **2** was coupled to carboxyl residues of the carrier protein keyhole limpet hemocyanin (KLH) using the water soluble coupling reagent 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), emulsified in Freund's complete adjuvant and used to immunized Balb/c mice. After three subsequent booster injections with KLH·**2** in Freund's incomplete adjuvant, spleens of mice were harvested and MAbs were produced using standard hybridoma methodology (5). Three MAbs (F123, F125, and F150) were selected in an immunoassay based on their selective binding to **2** coupled to bovine serum albumin (BSA). MAbs were purified by sheep anti-mouse affinity chromatography and dialyzed against PBS buffer, pH 7.4. Antibody concentration was determined by measuring optical density at 280 nm (where 1.2 absorbance units correspond to 1 mg mL^{-1} antibody in solution (6)) and using 190 kDa for the molecular weight of IgM monomer.

The three MAbs that showed selective binding to **2** were tested for catalytic activity against substrate **6** while monitoring the release of *p*-nitrophenolate. Of these, only F123 (an IgM monoclonal antibody as determined by isotyping) showed significant activity and was selected for further study. The F123-catalyzed reaction with **6** was shown to obey Michaelis–Menten kinetics with a K_m of 255 μM, and a k_{cat} of 0.01 min^{-1} (based on *p*-nitrophenol release and calculated on an active site basis). The observed rates were corrected for the background hydrolysis in buffer.

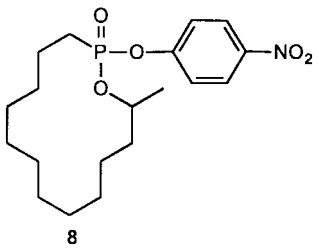


To illustrate that the activity of antibody F123 with **6** is not due to an esterolytic contaminant of the serum, an affinity purified control MAb raised against an unrelated hapten was run in parallel with F123. The control MAb showed no activity with substrate **6**. It should be noted that while this evidence is supportive, it is by no means definitive. In addition, substrate **7**, which lacks the secondary hydroxyl necessary for lactonization, was tested as a substrate for F123 and showed little or no *p*-nitrophenolate release compared with **6** (Fig. 1). This provided strong evidence that the catalytic MAb was not simply behaving as an esterase by hydrolyzing the *p*-nitrophenyl ester moiety. An alternate reason as to why **7** is not processed by F123 could simply be lack of recognition by the antibody. However, these two control experiments together provide substantial evidence that it is indeed the antibody that is carrying out the catalysis.

Fig. 1. Comparison of antibody F123 activity with hydroxy ester substrate **6** (○); with ester **7** (●). In each case, antibody was 0.46 μM; substrates were 100 μM; carried out in PBS buffer (pH 7.4) containing 10% DMSO and 0.5% Triton X-100; total reaction volume was 225 μL.



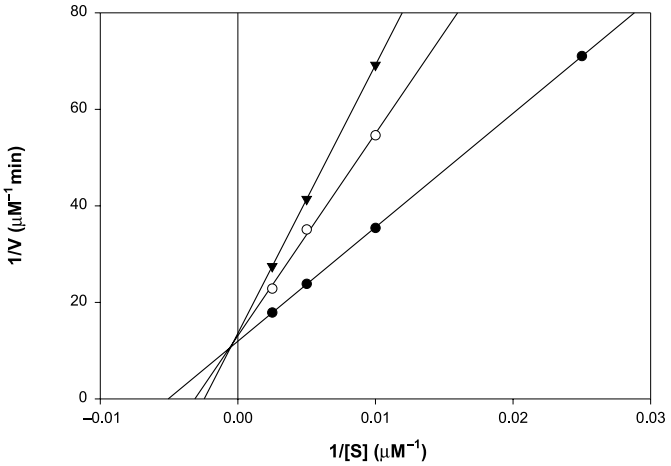
The 4-nitrophenyl phosphonate derivative **8** was synthesized by base hydrolysis of phenyl phosphonate **3** using refluxing 3 N NaOH–THF, producing the corresponding



phosphonic acid derivative in a 73% yield. Conversion of the phosphonic acid derivative to the acid chloride with oxalyl chloride, followed by coupling of this phosphoryl chloride intermediate with *p*-nitrophenol, gave a 1.2:1 ratio of diastereomers of **8** in 69% yield.

Owing to a lack of compound **2**, compound **8** was chosen for inhibition studies as we felt it would sufficiently mimic the putative transition state for the cyclization of substrate **6** to give lactone **1**. Inhibition assays with **8** (using a 1:1 mixture of diastereomers) were performed by varying the concentration of **8** at fixed levels of substrate **6** and MAb F123

Fig. 2. Inhibition of the F123-catalyzed macrolactonization of substrate **6** by a mixture of 4-nitrophenyl phosphonate diastereomers of compound **8**. The reactions were performed in PBS buffer (pH 7.4) containing 10% DMSO and 0.5% Triton X-100. The concentrations of compound **8** were: (●) 0 μM; (○) 2 μM; and (▼) 5 μM.



(Fig. 2). Unfortunately, accurate determination of K_i was precluded by the insensitivity of the assay employed. Concentrations of antibody in the same range as the apparent K_i value ultimately determined were needed to get measurable reaction rates. As a consequence the K_i value determined (K_i = 3 μM) can be regarded only as a rough estimate of the true K_i value. However, this result does clearly indicate that binding is tight and is fully consistent with the catalytic activity measured arising from the active site.

Lactone **1** was isolated from pooled catalytic experiments through ether extractions. Unambiguous identification of the lactone was carried out by comparison with authentic **1** (7) by GC, GC–MS, and HR-MS. No lactone was detected in the absence of antibody catalyst.

In summary, we have shown that monoclonal antibody F123, an IgM antibody, has catalyzed macrocyclic lactone ring formation. We feel this preliminary work opens the door for further potential methodology studies toward macrocycle synthesis.

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