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Syntheses and Reactions of the Diethyl α-Alkynylmalonates Involving the Generation of Conjugated Allenyl Esters as the Latent Active Species: A New Approach to the Development of Cysteine Proteinase Inhibitors

Yoshimitsu Nagao,* Kweon Kim, Shigeki Sano, Hisao Kakegawa,[†] Woo Song Lee, Hisashi Shimizu, Motoo Shiro,[††] and Nobuhiko Katunuma[†]*

Faculty of Pharmaceutical Sciences, The University of Tokushima, Sho-machi, Tokushima 770, Japan

[†]Institute for Health Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan

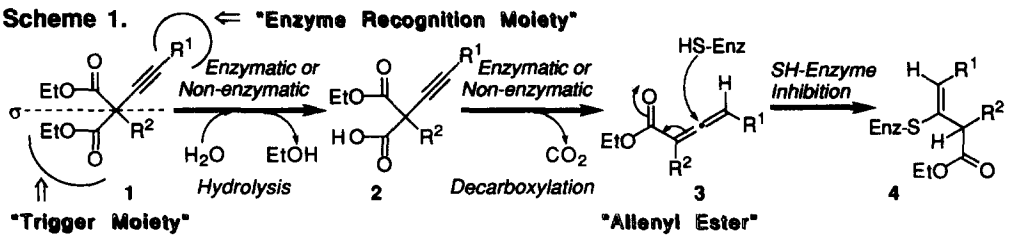
[††]Rigaku Corporation, 3-9-12 Matsubara-cho, Akishima-shi, Tokyo 196, Japan

Abstract: Various diethyl α-alkynylmalonates (DAM) having potential as cysteine proteinase inhibitors were synthesized by treatment of diethyl acetyliminomalonate (or ketomalonate) with several lithium acetylides. Hydrolytic decarboxylation of the DAM under the mild basic conditions afforded oxazole derivatives or allenyl esters which caused the Michael type reaction with EtSH.

Cysteine proteinases of the papain family have been implicated in the pathogenesis of a variety of serious diseases,[1] and the development of cysteine proteinase inhibitors has been extensively studied by Shaw,[1a,2] Krantz,[1b,3] Katunuma,[4] and Barrett groups,[5] independently. Katunuma and his colleagues have reported on the participation of cathepsin B in the antigen processing[6a] and the important role of cathepsin L in bone collagenolytic activity on bone resorption.[6b] Allenyl ketones from ynenol lactones inactivated a human leukocyte elastase.[3] Sugita et al. reported on the addition of thiols and amines to the conjugated allenyl ketones and esters.[7] The amino acids bearing an alkynyl group at the α-position of amino *N*-alkyl groups inhibited various enzymes (*e.g.*, alanine racemase,[8] GABA aminotransferase,[9] etc.) with the coenzyme vitamin B_6 in a suicide substrate manner.[10] A porcine liver esterase (PLE) hydrolyzed highly enantioselectively dimethyl α-methyl-α-pentadecanylmalonate to afford its chiral half methyl ester.[11] Ohta et al. reported a stereospecific decarboxylation of α-methyl-α-phenylmalonic acid with the enzyme in a microorganism.[12]

With the background described above and on the basis of a series of studies on enzymatic and non-enzymatic chiral induction onto σ-symmetric diesters and diamides,[13] we envisaged diethyl α-acetylamino(or methoxy)-α-alkynylmalonates (DAM) **1** as new lead compounds for developing cysteine proteinase inhibitors. Scheme 1 illustrates our idea of the latent function of DAM **1** against cysteine proteinases. Namely, enzymatic or non-enzymatic hydrolysis of an ethoxycarbonyl group (a trigger moiety leading **1** to the allenyl ester) of the DAM **1** followed by enzymatic or non-enzymatic decarboxylation of the resultant chiral half ester **2** may produce allenyl esters **3**. The allenyl esters **3** must be capable of trapping an active-site nucleophile (*i.e.*, SH group of the cysteine residue) of the target enzyme (**3** → **4**), which may cause inhibition of the SH enzymes. In the molecule of DAM **1**, R^1 group will have to be designed as the specific enzyme-recognition moiety bearing di- or tripeptide-containing substituent.[14]

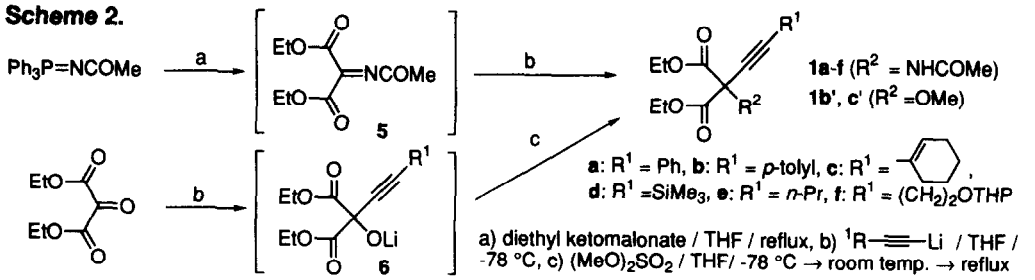
Scheme 1.



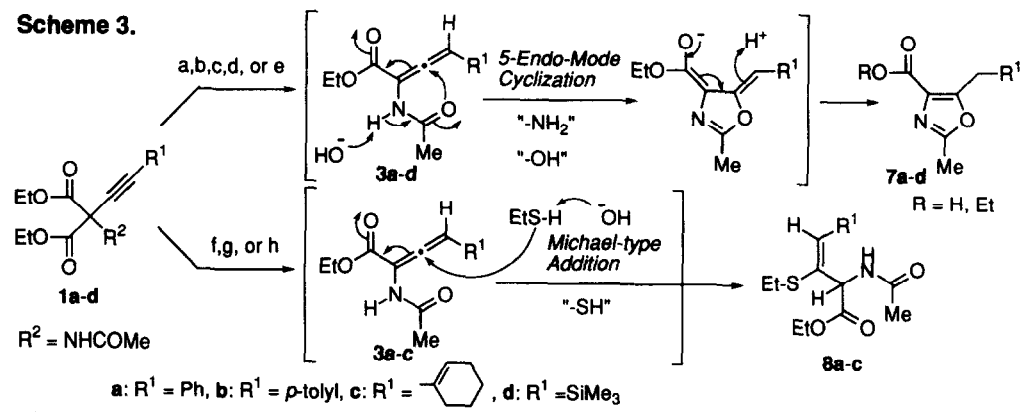
Thus, several DAM **1a-f** and **1b',c'** were readily derived from diethyl ketomalonate as follows (Scheme 2). A solution of (acetylimino)triphenyl phosphine (4.69 mmol)[15] and diethyl ketomalonate (4.69 mmol) in THF (40 ml) was refluxed for 10 h to generate the aza-Wittig product **5** and then cooled to -78 °C. To the solution, the lithium acetylide prepared by treating each corresponding alkynyl compound (4.69 mmol) with *n*-BuLi (4.69 mmol, 1.68 M soln in hexane) in THF (5 ml) was added dropwise at -78 °C with stirring over 1 h. After being stirred for 3 h, the reaction mixture was submitted to the usual work-up to give each DAM derivative **1a-f** in various yields [**1a**: oil (67%), **1b**: mp 132-133 °C (82%), **1c**: mp 82 °C (90%), **1d**: mp 97 °C (81%), **1e**: mp 53-54 °C (62%), **1f**: oil (74 %)]. DAM **1b',c'** (each pale yellow oil) were similarly obtained by treatment of diethyl ketomalonate (5 mmol) with lithium *p*-tolyl(or 1-cyclohexen-1-yl)acetylide (5 mmol) in THF (30 ml) at -78 °C for 30 min followed by methylation of the resultant lithiate **6** with dimethyl sulfate (5 mmol) under reflux for 3.5 h in 62% and 52% yields, respectively. The structures of **1a-f** and **1b',c'** were readily confirmed by their characteristic spectroscopic data.

Subsequently, we have investigated several chemical and enzymatic reactions of DAM **1a-d** in order to realize the rationality of our new idea depicted in Scheme 1. All results are summarized in Scheme 3. Treatment of **1b** (0.1 mmol) with porcine liver esterase (PLE) (Sigma Type I, 100 units)[13b] in 0.1M phosphate buffer (pH 7.5)-acetone (10 : 1) at 35-45 °C for 5 days gave an oxazole carboxylic acid **7b** (R = H) (mp 165 °C, in 83% yield as its methyl ester) instead of the half ester **2** (R^1 = NHCOMe, R^2 = *p*-Tolyl). The similar oxazole ring formation of **1a-d** readily proceeded not only in 0.1M phosphate buffer (pH 7.5) at 35-45 °C for 5 days without use of PLE but also in a solution of 1 mol eq. of KOH (1N aq. soln.) and EtOH at 0 °C for 7-15 min to give the corresponding ethyl esters **7a-d** (R = Et) in a range of 87-100% yields [**7a**: oil (98%), **7b**: oil (87% by phosphate buffer, 100% by 1N KOH), **7c**: mp 30-31 °C (97%), **7d** (R^1 = H): oil (97%)]. However, alkaline treatment of **1b** with EtONa or NaH in anhydrous THF at 0 °C for 2 h recovered the starting compound in 98 or 96% yield. The structure of oxazole carboxylic acid **7b** (R = H) was determined by the X-ray crystallographic analysis as shown in Fig. 1.[16] On treatment with excess EtBr in the presence of Et_3N in AcOEt-DMF (10 : 1), the carboxylic acid **7b** (R = H) was converted to its ethyl ester **7b** (R = Et) in 77% yield (Scheme 4). Similarly, hydrolysis of **7b** (R = Et) with KOH in aqueous EtOH gave **7b** (R = H) in 86% yield. This oxazole ring formation can be rationalized in terms of a rare intramolecular 5-endo-mode cyclization of acetylamino allenyl esters **3a-d** derived *in situ* from **1a-d** by their hydrolysis followed by decarboxylation of the resultant half ester **2** (R^1 = Ph, *p*-tolyl, etc., R^2 = NHCOMe). Actually, this particular intramolecular 5-endo-mode cyclization toward the oxazole ring prompted us to develop various endo-mode carbocyclic and heterocyclic cyclization reactions by utilizing the conjugated allenyl ketone system.[17] Compounds **1a-c** were allowed to react with 1 mol eq. of EtSH in the presence of 1 mol eq. of KOH (1N aq. soln.) in EtOH at 0 °C for 10 min. The Michael type addition of EtSH[18] to the allenyl esters **3a-c** generated *in situ*, proceeded to give the corresponding *E*-olefinic ("NOE experiment") thiol adducts **8a-c** in excellent yields [**8a**: mp 70 °C (97%), **8b**: mp 93.5 °C (96%), **8c**: oil (93%)] and in a highly stereoselective manner as expected (Scheme 3). Competitive intramolecular addition reaction onto **3b** among three kinds of nucleophiles [EtSH (2.4 mol eq.), an amine ($EtNH_2$ or imidazole, each 2.4 mol eq.) and EtOH (large excess)] was examined by employing **1b** in the same flask to give exclusively thiol adduct **8b** in 95 or 96% yield, respectively. Similar treatment of **1b** with an EtOH solution of $EtNH_2$ or imidazole without EtSH gave oxazole ester **7c** (R = Et) in 79 or 80% yield, respectively. Compounds **1b',c'** were similarly subjected to alkaline hydrolysis with 1 mol eq. of KOH (1N aq. soln.) at 0 °C for 10 min. In these cases, allenic esters **3b',c'** were obtained as an oily product in 94 or 72% yield, respectively. The structures of **3b',c'** were assigned by their characteristic IR [neat, ν 1932-1926 (allenic moiety) and 1733 (ester carbonyl) cm^{-1}] and ^1H NMR [200 MHz, $CDCl_3$, δ 7.08-6.75 (s, 1 H, allenic proton)] spectrum data. Treatment of **3b', c'** with 1.2 mol eq. of EtSH in the presence of 1 mol eq. of KOH (1N aq. soln.) in EtOH gave their thiol adducts **8b',c'** as an oil in 61 and 69% yields, respectively. We believe that DAM derivatives **1** (R^2 = NHCOMe) are promising chemoselective inhibitors against cysteine proteinases. In fact, the DAM derivatives **1a-c,e** and **1a',b'** exhibited significant inhibition[19,20] in their 10^{-5} M concentration order against hydrolysis of Z-Phe-Arg-MCA (MCA: methylcoumarylamino group) with human liver cathepsin B under the Barrett method.[21] Apparently, the enzyme inhibition with the allenic esters **3b',c'**

Scheme 2.

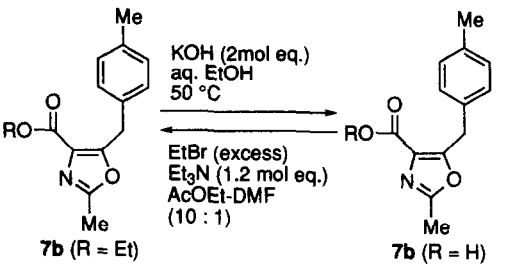


Scheme 3.



a) PLE / 0.1M phosphate buffer (pH 7.5)-acetone / 35-45 °C, b) 0.1M phosphate buffer (pH 7.5)-acetone / 35-45 °C, c) KE, d) $EtNH_2$ / KE, e) imidazole / KE, f) EtSH / KE, g) EtSH / $EtNH_2$ / KE, h) EtSH / imidazole / KE
KE = 1N KOH-EtOH / 0 °C

Scheme 4.



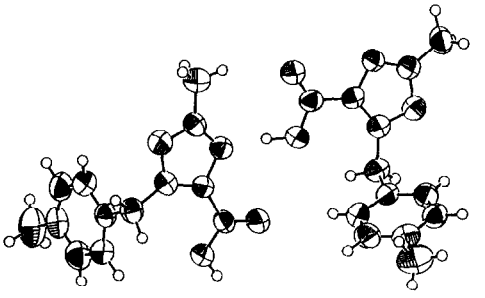


Fig. 1. X-ray analysis of **7b** (R = H)

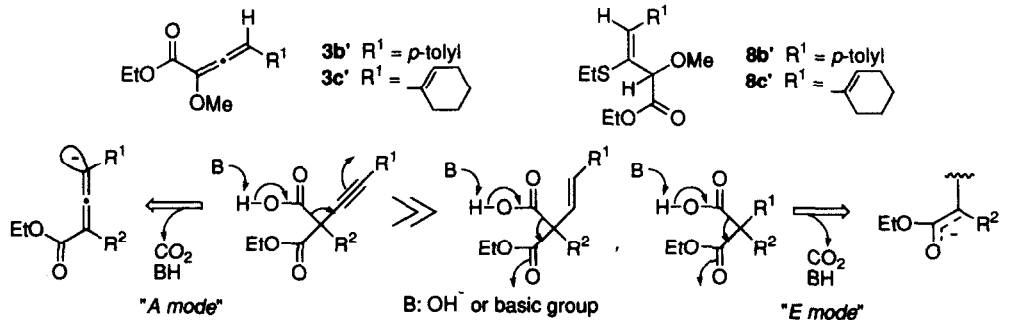


Fig. 2. Decarboxylation modes in the malonic half-esters

(**3b'**: 70.2% at 10^{-5} M, 98.5% at 10^{-4} M; **3c'**: 58.5% at 10^{-5} M, 84.9% at 10^{-4} M) proved to be stronger than that with their precursors **1b',c'** (**1b'**: 27.8% at 10^{-5} M, 50.6% at 10^{-4} M; **1c'**: 12.9% at 10^{-5} M, 38.9% at 10^{-4} M). Interestingly, compound **1b** (10^{-4}-10^{-6} M) does not inhibit the enzymatic hydrolysis of Suc-Leu-Leu-Val-Tyr- MCA with a serine proteinase, human pancreas chymotrypsin as we anticipated on the basis of the chemical reactions (Scheme 3).[19]

In general, decarboxylation of the malonic half-esters in the enolization mode (*E* mode in Fig. 2) seems to be difficult under the mild basic conditions.[22] However, decarboxylation of the α-alkynyl-malonic half-esters toward the conjugated allenyl ester formation (*A* mode in Fig. 2) must be easy by satisfaction with a stereoelectronic requirement due to the maximum overlap of the σ bond (R^2C-CO_2H) with the π bond of the alkynyl group. This new trigger system should be greatly suggestive for the fascinating bioorganic and chemical designs, some of which have been undertaken by Shibuya and Nagao groups.[23]

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