that proposed by Zielinska et al.[9] for the reaction of **1** with N_2O_5; however, in their case the loss of nitric acid is under thermodynamic control so that products with a substituent at the 3-position are not observed.

Thus, **1** allows a distinction between two different mechanisms of reaction of NO_2/N_2O_4. The isomer distribution in CH_2Cl_2 follows the electrophilic substitution pattern predicted from theory, whereas the product distribution in CCl_4 can best be rationalized by a radical mechanism for the nitration.[13] If 2NF is considered as a marker for radical nitration as we suggest, then the homolytic pathway, although not predominant, may be a minor path even in CH_2Cl_2 where some 2NF is formed.

Our results may be of environmental relevance since the uninitiated reaction of NO_2/N_2O_4 with **1** in a solvent of low polarity leads to the formation of nitroaromatics that are potential mutagens.[14] In this regard, 2NF was recently detected among the major nitro-PAH present in ambient air samples,[15-17] and atmospheric radical reactions of **1** initiated by N_2O_5 or hydroxyl radicals have been proposed as possible sources for this pollutant.[15-18]

Acknowledgment. This work was supported by a grant from the National Institutes of Health (HL-16029) and by a contract from the National Foundation for Cancer Research. We also thank David H. Giamalva for helpful suggestions.

(13) One of the referees suggested an alternative mechanism in which a cationic σ-complex is involved; however, we have preliminary data that show that nitrofluoranthene reacts with NO_2/N_2O_4 at a faster rate than does fluoranthene itself, excluding an electrophilic mechanism.

(14) Rosenkranz, H. S.; Mermelstein, R. *Mutat. Res.* **1983**, *114*, 217–267.
(15) Pitts, J. N., Jr.; Sweetman, J. A.; Zielinska, B.; Winer, A. M.; Atkinson, R. *Atmos. Environ.* **1985**, *19*, 1601–1608.
(16) Ramdahl, T.; Zielinska, B.; Arey, J.; Atkinson, R.; Winer, A. M.; Pitts, J. N., Jr. *Nature (London)* **1986**, *321*, 425–427.
(17) Sweetman, J. A.; Zielinska, B.; Atkinson, R.; Ramdahl, T.; Winer, A. M.; Pitts, J. N., Jr. *Atmos. Environ.* **1986**, *20*, 235–238.
(18) Arey, J.; Zielinska, B.; Atkinson, R.; Winer, A. M.; Ramdahl, T.; Pitts, J. N., Jr. *Atmos. Environ.* **1986**, *20*, 2239–2345.

Asymmetric Alkylation of Chiral β-Lactam Ester Enolates. A New Approach to the Synthesis of α-Alkylated α-Amino Acids

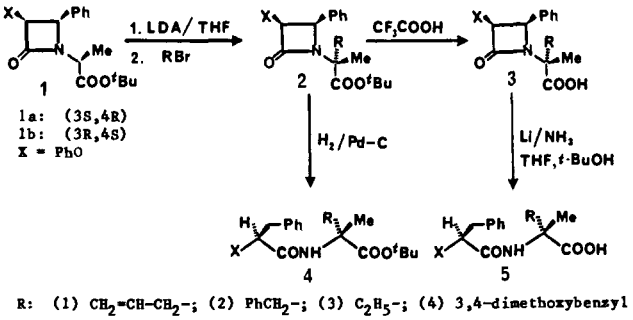
Iwao Ojima* and Xiaogang Qiu

Department of Chemistry
State University of New York at Stony Brook
Stony Brook, New York 11794

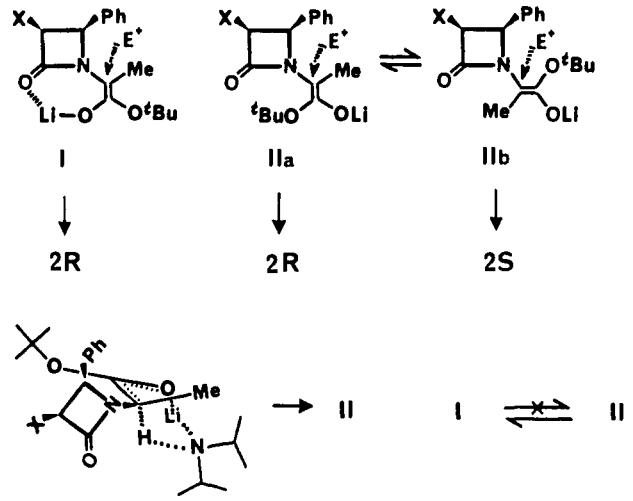
Received March 2, 1987

Asymmetric synthesis of nonproteinogenic amino acids with high optical purities has significant value since those amino acids can serve as valuable materials for the study of enzymic reaction mechanisms including enzyme inhibitors. Regarding this approach, Schöllkopf[1] and Seebach[2] reported methods based on bis(lactim) ethers and proline derivatives, respectively. Karady[3] and Williams[4] developed methods based on oxazolidinone and aza-δ-lactone, respectively. We would like to describe here novel approaches to this important synthetic problem through stereoselective alkylations of chiral β-lactam ester enolates followed by the reductive cleavage of the β-lactam rings.[5]

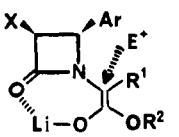
Scheme I



Scheme II



According to our hypothesis, the enolate would form a chelate with the β-lactam oxygen, and then electrophiles should attack from the side opposite to the 4-aryl group.



Thus, β-lactam enolate I was generated by treating β-lactam **1** with LDA (1.0 equiv) in THF at 0–5 °C, and the solution was cooled from −78 to −90 °C. The asymmetric alkylation was carried out by adding an alkyl halide to the enolate I. As shown in Table I, this new asymmetric alkylation proceeded with excellent stereoselectivity.[6] The reductive cleavage of the alkylated β-lactam ester **2** or carboxylic acid **3** thus obtained, through either hydrogenolysis on Pd–C or reduction with dissolving metal ($Li/NH_3/THF/t$-BuOH), gave the corresponding dipeptide derivatives **4** or **5** with high optical purity in excellent yields (Scheme I). The hydrolysis of **4** or **5** with 6 N hydrochloric acid in aqueous

(1) (a) Hartwig, W.; Schöllkopf, U. *Liebigs Ann. Chem.* **1982**, 1952–1970. (b) Groth, U.; Chiang, Y.-C.; Schöllkopf, U. *Ibid.* **1982**, 1756–1757. (c) Groth, U.; Schöllkopf, U.; Chiang, Y.-C. *Synthesis* **1982**, 864–866. (d) Groth, U.; Schöllkopf, U. Ibid. **1983**, 37–38.
(2) Seebach, D.; Boes, M.; Naef, R.; Schweizer, W. B. *J. Am. Chem. Soc.* **1983** *105*, 5390–5398.
(3) Karady, S.; Amato, J. S.; Weistock, L. M. *Tetrahedron Lett.* **1984**, *25*, 4337–4340.
(4) Sinclair, P. J.; Zhai, D.; Reibenspies, J.; Williams, R. M. *J. Am. Chem. Soc.* **1986**, *108*, 1103–1104.

(5) A review for the Pd-catalyzed hydrogenolysis of 4-aryl-β-lactams and its application to peptide synthesis, i.e., *β-Lactam Synthon Method*, see: (a) Ojima, I. In "Asymmetric Reactions and Processes in Chemistry", *ACS Symposium Series*; Eliel, E. L., Otsuka, S., Eds.; 1982; vol. 185, pp 109–138. For the application of this method to the syntheses of enkephalin analogues, labeled peptides, and other oligopeptides, see, for example: (b) Yamashita, M.; Abe, R.; Hatanaka, N.; Ojima, I. In *Peptide Chemistry 1982*; Sakakibara, S., Ed.; Protein Research Foundation: Osaka, Japan, 1983; pp 85–90. (c) Hatanaka, N.; Abe, R.; Ojima, I. *Chem. Lett.* **1982**, 445–448. (d) Ojima, I.; Shimizu, N. *J. Am. Chem. Soc.* **1986**, *108*, 3100–3102. (e) Ojima, I.; Chen, H.-J. C. *J. Chem. Soc., Chem. Commun.* **1987**, 625. (f) Ojima, I.; Nakahashi, K.; Brandstadter, S. M.; Hatanaka, N. *J. Am. Chem. Soc.* **1987**, *109*, 1798–1805.
(6) The absolute configurations of the newly formed chiral quaternary centers were determined based on the chemical correlation and their NMR spectra: for details, see Supplementary Material.

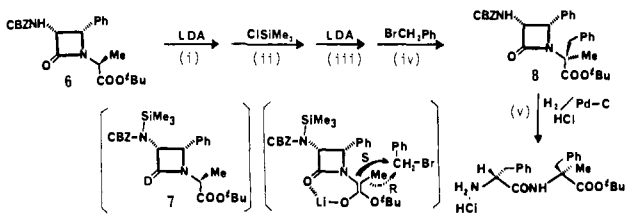
0002-7863/87/1509-6537$01.50/0

Table I. Asymmetric Alkylation of β-Lactam Esters **1**

β-lactam ester[a]	alkyl bromide	base[b]	genertn of enolate I temp (°C)	genertn of enolate I time (min)	additn of RBr temp (°C)	additn of RBr time (h)	product	yield[c] (%)	stereoselctvty[d] (% de)
1a	CH_2=CH—CH_2Br	LDA	0	15	-78	5	**2a-1**	95	>98 (R)
1a	CH_2=CH—CH_2Br	LHDS	0	15	0-5	5	**2a-1**	94	95 (R)
1b	CH_2=CH—CH_2Br	LDA	-78	15	-78	5	**2b-1**	95	34 (S)
1a	$PhCH_2Br$	LDA	0	15	-78	5	**2a-2**	96	>98 (R)
1a	$PhCH_2Br$	LDA	0	15	0-5	5	**2a-2**	95	93 (R)
1a	$PhCH_2Br$	LDA	-10	15	-10	5	**2a-2**	93	75 (R)
1a	$PhCH_2Br$	LDA	-90	15	-90	5	**2a-2**	95	50 (R)
1b	C_2H_5Br	LDA	0	15	-78	5	**2b-3**	95	>98 (R)
1b	3,4-$(MeO)_2$-C_6H_4-CH_2Br	LDA	0	15	0-5	5	**2b-4**	95	93 (R)

[a] **1a** = (3*S*,4*R*)-isomer; **1b** = (3*R*,4*S*)-isomer. [b] LDA = lithium diisopropylamide; LHDS = lithium hexamethyldisilylamide. [c] Determined by ^{1}H NMR. Conversion yield for the reaction is >99% in every case. [d] Determined by ^{1}H NMR. No the other diastereomer was detected for the cases with >98% de. *R* or *S* in the parentheses is the configuration of the newly formed quaternary center.

Scheme III[a]



[a] (i) 1 equiv, THF, -78 °C, 3 min; (ii) **1** equiv, -78 → 0 °C, 75 min; (iii) **1** equiv, THF, 0 °C, 10 min, then cool to -78 °C; (iv) 3 equiv, -78 °C (2 h), -78 → 0 °C (3 h), 0 °C (2 h), then saturated NH_4Cl in MeOH; SiO_2 column; (v) 10% Pd-C, 1 N HCl (1 equiv), MeOH, 50 °C, 12 h.

THF at 110 °C gave the corresponding optically pure α-amino acid in good yield.[6]

In this asymmetric alkylation, we observed an interesting dependence of stereoselectivity on the reaction temperature as shown in Table I. When the reaction was carried out at -78 to -95 °C, the results of the alkylations were discouraging since the ratios of two diastereomers were only 2:1-3:1, and the enolate generated showed intense violet color. However, when the enolate was generated at 0-5 °C, the stereoselectivities of the alkylations were excellent, and the enolate generated exhibited yellow color.

Upon treatment with LDA or LHDS, a β-lactam ester, e.g., **1a**, should generate a chelating enolate I and/or a nonchelating enolate II. On the basis of the widely accepted transition-state model for the kinetic enolate formation, the nonchelating enolate II is favorable, when generated at -78 to -90 °C (Scheme II). Since the kinetic enolate cannot form a rigid chelate ring with the β-lactam oxygen by any means, it is reasonable that the stereoselectivity of the alkylation is low. The experiments at 0-5 °C imply that the thermodynamic enolate I which has a rigid chelate structure is generated at this temperature as originally designed and achieves excellent stereoselectivity. Thus, there is an isomerization process from the kinetic enolate II to the thermodynamic enolate I when the reaction is carried out at 0-5 °C. In fact, we observed a short-lived violet color at 0 °C when LDA in THF was added dropwise to a solution of β-lactam ester 1a in THF.

When 3-CBZ-NH-β-lactam ester **6** (CBZ = carbobenzyloxy) was employed as a substrate for the asymmetric alkylation, the reaction using 2 equiv of LDA and 1 equiv of benzyl bromide gave a poor result (ca. 20% de). This may indicate that the β-lactam oxygen cannot hold double coordination of lithium. Accordingly, chlorotrimethylsilane (TMS-Cl) was added after the addition of 1 equiv of LDA at -78 °C to form 3-CBZ-N(TMS)-β-lactam ester **7**, and then another 1 equiv of LDA was added at 0 °C followed by the addition of benzyl bromide at -78 °C. The stereoselectivity of this reaction was 14:1 as we expected (Scheme III). The hydrogenolysis of the alkylated β-lactam ester **8** on Pd-C gave (*R*)-(phenylalanyl)-(*S*)-2-methylphenylalanine *tert*-butyl ester hydrochloride in nearly quantitative yield.

Further studies on the application of this method to new double and triple asymmetric alkylations are actively in progress.

Acknowledgment. This research was supported by grants from the Center for Biotechnology, SUNY at Stony Brook, which is sponsored by the New York State Science and Technology Foundation and from National Institutes of Health (NIGMS). The generous support from Ajinomoto Co., Inc. is also gratefully acknowledged.

Supplementary Material Available: Procedures for the asymmetric alkylation of β-lactams and for the reductive cleavage of alkylated β-lactam esters, identification data for the alkylated β-lactam esters, the determination of absolute configurations, and a stereomodel of a lithium β-lactam ester enolate (5 pages). Ordering information is given on any current masthead page.

Catalysis by Human Leukocyte Elastase. 9. pH-Dependent Change in the Rate-Limiting Step[1,2]

Ross L. Stein*[3] and Anne M. Strimpler

Department of Pharmacology, Stuart Pharmaceuticals, a Division of ICI Americas Inc. Wilmington, Delaware 19897

Received June 12, 1987

We report that the rate-limiting step of k_c (see Scheme I and eq 1-4) for the HLE-catalyzed[4] hydrolyses of specific peptide *p*-nitroanilide substrates is dependent on pH and changes from acylation, at pH values less that 5.5, to deacylation, at pH values greater than 6.0.

$$k_c = \frac{k_2 k_3}{k_2 + k_3} \quad (1)$$

$$K_m = K_s \frac{k_3}{k_2 + k_3} \quad (2)$$

$$K_s = (k_{-1} + k_2)/k_1 \quad (3)$$

$$k_c/K_m = k_2/K_s = \frac{k_1 k_2}{k_{-1} + k_2} \quad (4)$$

pH dependencies for the HLE-catalyzed hydrolyses of four *p*-nitroanilides (Table I) indicate that while the pK_a for k_c/K_m

(1) For part 8 in this series, see ref 2.
(2) Stein, R. L.; Strimpler, A. M. *Biochemistry* **1987**, *26*, 2238-2242.
(3) Present address: Department of Enzymology, Merck Institute for Therapeutic Research, P.O. Box 2000, Rahway, NJ 07065.
(4) Abbreviations: HLE, human leukocyte elastase; MeOSuc, methoxysuccinyl; *p*NA, *p*-nitroanilide.

0002-7863/87/1509-6538$01.50/0