synthesis of these derivatives often necessitates the regiochemically controlled boron tribromide mediated opening of the nonsymmetric 1,4-ether. The selective ether-bond cleavage at the benzylic carbon in the bay region followed by the stereocontrolled introduction of the bromine atom at the carbon would give a bromo alcohol (e.g., 13) ideally suited for the synthesis of the bay-region diol epoxides. In order to address this regioselectivity issue, the BBr3-mediated ether opening of the readily available nonsymmetric phenanthrene derivatives 12 was first examined. Contrary to the anticipated contribution of the more stable bay-region benzylic carbocation character<sup>2</sup> to the transition state in the ether ringopening reaction, treatment of diacetate 12a<sup>9</sup> with BBr<sub>3</sub> at 0 °C resulted in the exclusive formation of bromo alcohol 14a (95%) with virtually no formation of the desired regioisomer 13a. Notably, the bromine atom in 14a was introduced with overall retention of the configuration in the reaction (see 11). In an attempt to reverse this regiochemical selectivity for the ether-ring opening with BBr<sub>3</sub>, cyclic carbonate 12b, prepared from 12 (R = H) with N, N'-carbonyldiimidazole in 98% yield, was subjected to the above BBr<sub>3</sub> conditions at -20 °C. This gave preferentially the desired bay-region bromide 13b (75%) with overall retention of stereochemistry along with regioisomer 14b (16%). While a mechanistic rationale for this observed reversal in regioselectivity of the ether-ring opening remains ambiguous, it may be reasonable to assume that 12b, unable to provide a direct anchimeric assistance by the carbonate group, may be opened preferentially to the more stable bay-region benzylic carbocation intermediate. This intermediate is likely to adopt a half-chair conformation in the transition state, as indicated in 15 (R = H), with a bromine anion



approaching from the axial direction due to the steric congestion imposed by the bay-region aromatic hydrogen, thus providing bromo alcohol 13b with overall retention of stereochemistry at the bay-region benzylic carbon. Bromo alcohol 13b was subsequently converted into syn and anti bay-region diol epoxides, 1,2-trans-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrophenanthrenes, in three [(i) Cr(II);<sup>6</sup> (ii) NBA/20% aqueous THF; (iii) KO-t-Bu/THF; 66% overall yield] and one (0.5 M NaOH/50% aqueous dioxane; 83% yield) steps, respectively.

The methodology established above was next applied to the synthesis of the putative metabolites 1a, 1b, and 1c of the carcinogen 1,4-dimethylphenanthrene. The requisite cyclic carbonate 16 was obtained in overall 50% yield from 1-(tosyloxy)-2-bromo-5,8-dimethylnaphthalene<sup>10</sup> through its initial 1-naphthyne reaction with 4 (R = Bn) followed by catalytic hydrogenation of the cycloadduct, removal of the benzyl group, and cyclic carbonate formation. Treatment of 16 with BBr<sub>3</sub> (3.0 equiv) at -40 °C resulted in the smooth, exclusive formation of the desired bromo alcohol 17 in 83% yield. Reductive elimination of the bromo carbonate unit in 17 with Cr(ClO<sub>4</sub>)<sub>2</sub> produced 7,8-*trans*-dihydrodiol 1c in 81% yield. Treatment of the bromo hydrin produced from 1c (NBA/20% aqueous THF, 0 °C, 3 h) with KO-

*t*-Bu/THF, 0 °C, for 1 h afforded *syn*-diol epoxide 1a (mp 145-146 °C) in 75% overall yield from 1c. The formation of the anti isomer 1b from 17 proved to be problematic. The use of the aqueous basic conditions that were effective in similar cases resulted in the clean formation of the hydrolysis product of the epoxide, i.e.,  $(\pm)$ -5 $\beta$ , $6\alpha$ , $7\alpha$ , $8\beta$ -tetraol. This problem of hydrolysis was circumvented by the use of the two-phase, aqueous base/THF system for the reaction. Thus, treatment of bromo alcohol 17 with 4.0 M NaOH/THF (1/20) at room temperature for 20 min produced the desired *anti*-diol epoxide 1b (mp 151-152 °C) in 93% yield. Preliminary biological studies indicate that these two diol epoxides 1a and 1b are potent mutagens.<sup>11</sup>

In conclusion, the novel methodology described above should have general applicability for the synthesis of biologically important bay-region diol epoxide and *trans*-dihydrodiol metabolites of various carcinogenic PAHs. In particular, the unique two-phase, aqueous NaOH/THF conditions may offer a valuable solution to the synthesis of bay-region *anti*-diol epoxides.

Acknowledgment. We thank the National Institutes of Health (CA 28158) for generous financial support of this work.

Supplementary Material Available: Experimental details for the synthesis of 1a, 1b, and 1c and spectroscopic and microanalytical data for these and their synthetic intermediates (11 pages). Ordering information is given on any current masthead page.

(11) Sinsheimer, J. E.; Giri, A. K.; Messerly, E. A.; Jung, K.-Y.; Koreeda, M. Carcinogenesis 1989, 10, 1123.

## Urethane-Protected Amino Acid N-Carboxy Anhydrides and Their Use in Peptide Synthesis

William D. Fuller,\* Michael P. Cohen, Mitra Shabankareh, and Robert K. Blair

BioResearch, Inc., 11189 Sorrento Valley Road #4 San Diego, California 92121

Murray Goodman

Department of Chemistry, University of California, San Diego La Jolla, California 92093

Fred R. Naider

Department of Chemistry, College of Staten Island of the City University of New York 130 Stuyvesant Place, Staten Island, New York 10301

Received May 2, 1990

We report the general synthesis of novel urethane-protected amino acid N-carboxy anhydrides (UNCAs, I) and their use in peptide synthesis. We have prepared many of the [(9fluorenylmethyl)oxy]carbonyl (Fmoc), benzyloxycarbonyl (Z), and *tert*-butyloxycarbonyl (Boc) protected amino acid NCAs. These compounds are stable (in the absence of water), crystalline solids. They are highly reactive toward nucleophiles and form peptide bonds quickly and cleanly with carbon dioxide as the only coproduct.



Several researchers have attempted to use amino acid *N*-carboxy anhydrides (NCAs) in stepwise polypeptide synthesis.<sup>1</sup> However,

<sup>(9)</sup> Obtained in four steps from 1-bromo-2-(tosyloxy)naphthalene in 53% overall yield following the identical sequence used for the synthesis of 6. (10) Jung, K.-Y.; Koreeda, M. J. Org. Chem. 1989, 54, 5667.

NCAs exhibit poor stability<sup>2</sup> and often give multiple additions during each coupling cycle.<sup>3</sup> Several investigators have recognized the potential usefulness of substituted NCAs<sup>4</sup> and particularly urethane-protected NCAs. Kricheldorf<sup>5</sup> prepared N-(methoxycarbonyl)glycine-NCA and N-(ethoxycarbonyl)glycine-NCA by phosgenation of trimethylsilyl amino acid esters. He concluded that any urethane-protected amino acid NCA containing a side chain rather than the hydrogens of a glycine residue or a urethane larger than ethyl could not be prepared by this approach. Block and Cox<sup>4b</sup> also reported unsuccessful attempts to prepare Nbenzyloxycarbonyl or N-tert-butyloxycarbonyl NCAs by phosgenation of the urethane-protected amino acid. Halstrom<sup>4</sup> reiterates the conclusion that, in general, urethane-protected amino acid N-carboxy anhydrides cannot be prepared.

The synthesis of UNCAs<sup>6</sup> was achieved by the condensation of acylating reagents (acyl halides, chloroformates, anhydrides, etc.) with NCAs in aprotic solvents such as THF, EtOAc, or CH<sub>2</sub>Cl<sub>2</sub> in the presence of N-methylmorpholine, a base that does not readily polymerize or ring-open the NCAs. The crude product may be reprecipitated from ethyl ether/hexane and, in most cases, crystallized from an appropriate solvent such as CCl<sub>4</sub>, diisopropyl ether, diethyl ether, or cyclohexane. The structures of the UNCAs were supported by 360-MHz <sup>1</sup>H NMR spectroscopy and, in the case of Fmoc-O-tert-butylthreonine-NCA, x-ray crystallography. The pure UNCAs gave the expected analytical results (IR, <sup>1</sup>H NMR, gel permeation chromatography, and elemental analysis). (See Table I.)

Urethane-protected amino acid NCAs are highly effective reagents for peptide synthesis. Addition of the first protected amino acid to a hydroxyl-containing resin was achieved using FMOC amino acid NCAs in 30-60 min with no detectable racemization (<0.1%). Addition of an UNCA to the deprotected amine terminus of the peptide occurs in 15-45 min, in a variety of aprotic solvents, in high yields and without detectable racemization. For example, Fmoc-L-leucine-NCA (3-fold excess) reacts with 4-alkoxy-2',4'-dimethoxybenzhydrol on 2% cross-linked polystyrene resin<sup>7</sup> by being shaken for 45 min in toluene in the presence of 0.02 equiv of N-methylmorpholine. Fmoc-Leu-O-resin with a substitution of 0.4 meq/g was obtained after filtration, washing, and drying under high vacuum. The absence of racemization was established by standard solid-phase procedures.<sup>8</sup>

(4) (a) Halstrom, J.; Christensen, T.; Brunfeldt, K. Hoppe-Seyler's Z.
Physiol. Chem. 1976, 357, 999. (b) Block, H.; Cox, M. E. In Pept. Proc. 5th Eur. Symp.; Young, G. T., Ed.; MacMillan: New York, 1963; pp 83-87. (c)
Akiyama, M.; Hasegawa, M.; Takeuchi, H.; Shimizu, K. Tetrahedron Lett. (5) Kricheldorf, H. R. Makromol. Chem. 1977, 178, 905.

ACYL CARRIER DECAPEPTIDE (65-74)





Figure 1. HPLC profile of crude acyl carrier peptide (65-74 VQAAI-DYING). HPLC conditions: column, Vydac RP-18 (4.6 × 250 mm); solvents (A) 0.05% TFA in water and (B) 0.05% TFA in CH<sub>3</sub>CN; flow, 1.0 mL/min; linear gradient from 0 to 60% B over 35 min.

Fmoc-amino acid NCAs were used to prepare acyl carrier peptide (65-74, VQAAIDYING) in a peptide synthesis flow reactor.<sup>9</sup> After cleavage from the resin, the crude peptide was obtained in 73% yield. The synthetic protocol and the reversedphase HPLC are shown in Figure 1. The high purity of this crude decapeptide clearly demonstrates the utility of urethane-protected NCAs in peptide synthesis.

Urethane-protected N-carboxyl anhydrides represent a new, broadly applicable, stable class of protected and activated derivatives of amino acids which can be used directly for forming peptide bonds. They also provide the added advantage of liberating carbon dioxide as the only coproduct, which is innocuous. Urethane-protected NCAs can be used in solid-phase flow and batch reactors or in solution coupling reactions. We believe that these

<sup>(1)</sup> Katakai, R. G. J. Org. Chem. 1975, 40, 2697 and references cited therein

<sup>(2)</sup> Kricheldorf, H. R. a-Aminoacid-N-Carboxy-Anhydrides and Related

<sup>(2)</sup> Kneihoff, H. K. & Amindacia-IV-Carboxy-Anayarides and Related Heterocycles: Syntheses, Properties, Peptide Synthesis, Polymerization; Springer-Verlag: Berlin, 1987; pp 22-23.
(3) (a) Bailey, J. L. J. Chem. Soc. 1950, 3461. (b) Bartlett, P. D.; Jones, R. H. J. Am. Chem. Soc. 1957, 79, 2153. (c) Denkewalter, R. G.; Schwam, H.; Strachan, R. G.; Beesley, T. E.; Veber, D. F.; Schoenewaldt, E. F.; Barkemeyer, H.; Paleveda, W. J., Jr.; Jacob, T. A.; Hirschmann, R. J. Am. Chem. Soc. 1966, 29, 2163. (d) Hirschmann, P. Stepcher, P. G.; Schwam. Chem. Soc. 1966, 88, 3163. (d) Hirschmann, R.; Strachan, R. G.; Schwam, H.; Schoenewaldt, E. F.; Joshua, H.; Barkemeyer, B.; Veber, D. F.; Paleveda, W. J., J.; Jacob, T. A.; Beesley, T. E.; Denkewalter, R. G. J. Org. Chem. 1967, 32, 3415

<sup>(6)</sup> Standard procedure for the preparation of Fmoc-amino acid NCAs: An amino acid NCA (20 mmol) was dissolved in THF (67 mL, dried over 4 Å molecular sieves) under N2 and cooled with stirring to about 2 °C in an ice bath. Fmoc-Cl (22 mmol) was added all at once, followed by the slow addition of dry N-methylmorpholine (29 mmol). The resulting suspension was stirred for 2 h at 2-5 °C, after which time 4 M HCl in dioxane was added slowly until a pH of 4-5 (sample diluted in water) was obtained. The solids were removed by filtration and washed with dry THF. The combined THF were removed by filtration and washed with dry THF. The combined THF solutions were concentrated under reduced pressure and the resulting oil dissolved in a minimum volume of dry diisopropyl ether. Dry hexane was added to the cloud point, and the solution was kept at -20 °C overnight. The resulting crystalline product was collected by filtration, washed well with dry hexane, and dried under high vacuum. Yields were typically 50-70%. All solvents were dried by being passed through a column of 4 Å molecular sieves. (7) Rink, H. *Tetrahedron Lett.* 1987, 28, 3787. (8) Merrifield, R. B. J. Am. Chem. Soc. 1963, 85, 2149. For a review, see: Barany, G.; Merrifield, R. B. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 2, p 1.

<sup>(9)</sup> For the preparation of acyl carrier peptide (65-74, VQAAIDYING) in a flow reactor, a column was charged with Fmoc-glycine resin (0.8 g, 0.36 meq/g) and equilibrated with dry DMF at a flow rate of 11 mL/min. The The resin was allowed to react sequentially with a 3-fold excess of each of the Fmoc-amino acid NCAs according to the following protocol: (1) deprotect (10% piperidine in DMF), 10 min at 11 mL/min; (2) wash (DMF), 5 min at 11 mL/min; (3) couple (0.15 M UNCA in DMF), 45 min at 11 mL/min; (4) wash (DMF), 5 min at 11 mL/min; (5) repeat steps 1-4. Fmoc-O-tert-(4) Wash (DMP), 5 min at 11 mic/min (5) repeat steps 1-4. Finor-outer-butyltyrosine was coupled via the BOP reagent (ref 10) in order to demon-strate the compatibility of the Fmoc NCA procedure with standard peptide synthesis procedures. After deprotection of the terminal protecting group, the resin was removed from the column, washed with dry CH<sub>2</sub>Cl<sub>2</sub>, and dried under high vacuum. The peptide was cleaved from the resin by treatment for 1 h with 50% TFA in dry  $CH_2Cl_2$  containing 2.5% anisole and 2.5% pentamethylbenzene. After removal of solvents under reduced pressure and lyo-philization, crude acyl carrier peptide (65-74) was obtained in 73% yield. All solvents were dried by being passed through a column of 4Å molecular sieves. Amino acid anal. Calcd for VQAAIDYING: V = 1.00, Q = 1.00, A = 2.00, I = 2.00, D + N = 2.00, Y = 1.00, G = 1.00. Found: V = 1.00, Q = 1.02, A = 2.08, I = 2.05, D + N = 2.20, Y = 1.05, G = 1.00. FAB MS (M + H)<sup>+</sup> calcd for VQAAIDYING 1064, found 1064. (10) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. Tetrahedron Lett. 1975,

<sup>1219</sup> 

7416

amino acid	urethane	mp, °C	$[\alpha]^{25}$ <sub>D</sub> , deg
L-Ala	Fmoc	106-107	+28.7
D-Ala	Fmoc	109-113 dec	-28.7
L-Asn(trityl)	Fmoc	134-137	+29.1
L-Asp( <i>β-tert</i> -butyl)	Fmoc	65-70 dec	+22.4
L-Gln(trityl)	Fmoc	123-126	+19.4
$L$ -Glu( $\gamma$ -tert-butyl)	Fmoc	120-123	+29.3
Gly	Fmoc	156-157 dec	00.0
L-Île	Fmoc	117-118	+25.9
L-Leu	Fmoc	118-120	+38.0
L-Lys(e-Boc)	Fmoc	81-85	+25.3
L-Met	Fmoc	74-75	+69.3
L-Phe	Fmoc	59-61	+101.9
L-Ser(O-tert-butyl)	Fmoc	54-57	+27.5
L-Thr(O-tert-butyl)	Fmoc	124-127	+31.2
L-Trp(N <sup>in</sup> -formyl)	Fmoc	108 dec	87.9
L-Tyr(O-tert-butyl)	Fmoc	122-124	+110.6
L-Val	Fmoc	83.5-87	+14.8
L-Ala	Boc	103-104.5	+21.6
L-Ser(O-benzyl)	Boc	98-99.5	+47.2
D-Ala	Z	103-104.5	-52.1
L-Phe	Z	105-106	+127.6

reagents will greatly facilitate and enhance the scope of peptide synthesis.

Acknowledgment. We are pleased to acknowledge Mr. Adam D. Mann, Mr. David T. Jones, and Dr. Sherrell S. Early for their technical support and Dr. Raj. K. Chadha for X-ray crystallography.

Supplementary Material Available: Analytical data (mp, IR, <sup>1</sup>H NMR, CHN analysis, optical rotation) for all compounds listed in Table I, FAB mass spectrum of crude acyl carrier peptide (65-74), and crystallographic structure determination summary, experimental procedures, data collection, data reduction, structure solution and refinement, tables of general temperature factor expressions and torsional angles, and drawings and unit cell packing diagram of Fmoc-O-tert-butylthreonine-NCA (27 pages); listing of observed and calculated structure factors of Fmoc-Otert-butylthreonine-NCA (7 pages). Ordering information is given on any current masthead page.

## Synthesis of Dynemicin A Models

K. C. Nicolaou,\* C.-K. Hwang, A. L. Smith,<sup>†</sup> and S. V. Wendeborn<sup>‡</sup>

> Department of Chemistry Research Institute of Scripps Clinic 10666 North Torrey Pines Road La Jolla, California 92037 Department of Chemistry University of California, San Diego La Jolla, California 92093

Received May 18, 1990

Dynemicin A (1, Scheme I) is a potent antibacterial and anticancer agent recently isolated from Micromonospora chersina.1 Its striking molecular structure combines characteristics of both the enediyne<sup>2,3</sup> and the anthracycline<sup>4</sup> classes of antibiotics and Scheme I. Structure of Dynemicin A (1) and Retrosynthetic Disconnection of Model Systems 2 and 3



presents a considerable challenge to organic synthesis as well as a unique opportunity for the development of new synthetic technology and therapeutic agents. In this communication we report the synthesis, crystal structures, and Bergman-type cyclizations of two novel dynemicin A models (2 and 3, Scheme I) containing the nitrogen, epoxide, and enediyne functionalities of the natural product.

The retrosynthetic analysis that led to the present synthetic strategy is outlined in Scheme I (2,  $3 \rightarrow 4$ ). Scheme II<sup>5</sup> summarizes the construction of 2 and 3 starting from quinoline derivative 4. Thus treatment of 46 with mCPBA in dichloromethane gave the corresponding N-oxide, which underwent regiospecific rearrangement<sup>7</sup> upon heating in acetic anhydride to give the acetoxy derivative 5 (62% overall yield). This was converted to the corresponding silvl ether 7 in 92% overall yield by standard methods via hydroxy compound 6. Addition of phenyl chloroformate<sup>8</sup> to a mixture of compound 7 and ethynylmagnesium bromide at -78 °C led to the formation of compound 8 in 92% yield.<sup>9</sup> Treatment of 8 with mCPBA led to epoxide 9 (85%),<sup>10</sup> which was converted to ketone 11 via alcohol 10 by desilylation followed by oxidation (79% overall). Coupling 11 with vinyl

(5) All new compounds exhibited satisfactory spectral and analytical and/or exact mass data. Yields refer to chromatographically and spectroscopically homogeneous materials.

(6) (a) Masamune, T.; Takasugi, M.; Suginome, H.; Yokogama, M. J. Org. Chem. 1964, 29, 681-685. (b) Curran, D. P.; Kuo, S.-C. J. Org. Chem. 1984, 49, 2063-2065. (c) Hollingsworth, B. L.; Petrow, V. J. Org. Chem. 1948, 13, 1537-1541.

(7) Bockelheide, N.; Linn, W. J. J. Am. Chem. Soc. 1954, 76, 1286-1291.
(8) Comins, D. L.; Myoung, Y. C. J. Org. Chem. 1990, 55, 292-298.
(9) Compounds 8-10 exhibited two sets of <sup>1</sup>H and <sup>13</sup>C NMR signals (ca. 3:1 ratio), due to the presence of two isomers. This phenomenon disappeared, as expected, upon arrival at intermediate 11 as evidenced by NMR spectrometers. troscopy

(10) The stereochemistry of the epoxide functionality in this compound was tentatively assigned as shown and was confirmed by its subsequent conversion into 2

<sup>&</sup>lt;sup>†</sup>Recipient of a NATO (SERC, U.K.) Postdoctoral Fellowship, 1990–1992. <sup>‡</sup>Recipient of a Verband Der Chemischen Industrie Doctoral Fellowship, 1989-1990

<sup>(1) (</sup>a) Konishi, M.; Ohkuma, H.; Tsuno, T.; Oki, T.; VanDuyne, G. D.; Clardy, J. J. Am. Chem. Soc. 1990, 112, 3715-3716. (b) Konishi, M.;
 Ohkuma, H.; Matsumoto, K.; Tsuno, T.; Kamei, H.; Miyaki, T.; Oki, T.;
 Kawaguchi, H.; VanDuyne, G. D.; Clardy, J. J. Antibiot. 1989, 42, 1449-1452. (c) Sugiura, Y.; Shiraki, T.; Konishi, M.; Oki, T. Proc. Natl. Acad. Sci. U.S.A. 1990, 87, 3831-3835.

<sup>(2)</sup> Calicheamicins: (a) Lee, M. D.; Dunne, T. S.; Siegel, M. M.; Chang, C. C.; Morton, G. O.; Borders, D. B. J. Am. Chem. Soc. **1987**, 109, 3464-3466. (b) Lee, M. D.; Dunne, T. S.; Chang, C. C.; Ellestad, G. A.; Siegel, M. M.; Morton, G. O.; McGahren, W. J.; Borders, D. B. J. Am. Chem. Soc. 1987, 109, 3466-3468.

<sup>(3)</sup> Esperamicins: (a) Golik, J.; Clardy, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Doyle, T. W. J. Am. Chem. Soc. 1987, 109, 3461-3462. (b) Golik, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohjuma, H.; Doyle, T. W. J. Am. Chem. Soc. 1987, 109, 3462-3464.

<sup>(4) (</sup>a) Anthracycline Antibiotics; El Khadem, H. S., Ed.; Academic Press: New York 1982. (b) Recent Aspects in Anthracyclinone Chemistry; Tetrahedron Symposia-in-Print No. 17, Kelly, T. R., Ed.; Tetrahedron 1984, 40, 4537-4794.