the symmetric anhydride of the Boc-amino acid was filtered directly into the NBH-resin (1 g) swelled in $CH_2Cl_2$ (10 mL). Pyridine (0.4 mL) was added and the mixture was stirred at room temperature for 24 h. The resin was filtered, washed with $CH_2Cl_2$ (10 × 3 × 2 min) and methanol (10 mL × 3 × 2 min), and dried in vacuo. The capacity of the resin was determined by amino acid analysis. The acid stability of the NBH-resin ester linkage was studied by using Boc-Gly-NBH-resin (**5f**) as the test sample. Boc-Gly-NBH-resin (200 mg) in 5 mL of 4 N HCl–dioxane, 30 TFA–$CH_2Cl_2$, or 50% TFA–$CH_2Cl_2$ was stirred separately for definite intervals and the amount of amino acid remaining in the resin was determined (Figure 2).

**Peptide Synthesis Using NBH-resin. General Procedure.** All the Boc-amino acids were coupled to the NBH-resin by the symmetric anhydride method using a 1.5-fold molar excess of DCC and a 3-fold molar excess of Boc-amino acids except for Boc-Pro-OH where a 6-fold molar excess was used. The coupling time for the first amino acid was 6 h. A second coupling was performed for 1 h to ensure maximum incorporation of the first amino acid. The subsequent amino acids were coupled for 3 h. The Boc group was removed with 4 N HCl–dioxane (10 mL/g) for 30 min; 10% $Et_3N$ in $CH_2Cl_2$ was used for neutralization. Each step of the coupling was followed by the semiquantitative ninhydrin test. Using these procedures Boc-Pro-Gly-NBH-resin (**6**), Boc-Ala-Gly-Val-NBH-resin (**8**), and Boc-Leu-Ala-Gly-Val-NBH-resin (**9**) were synthesized (Scheme II).

**General Procedure for the Photolytic Cleavage of the Resin-Bound Peptides.** The peptide resin (1 g) was suspended in anhydrous ethanol (150 mL) in a water-cooled immersion-type photochemical irradiator. The suspension was deaerated with dry $N_2$ gas for 1 h and irradiated with a philips HPK, 125-W mercury lamp at 350 nm, under gentle magnetic stirring. A 40% solution of $CuSO_4$ was used to filter out light waves below 320 nm. After irradiation for 20–24 h, the resin was filtered and washed with ethanol, methanol and $CH_2Cl_2$. The solvent was evaporated from the combined filtrate and washings in a vacuum rotary evaporator. The crude products were purified by chromatography and characterized by amino acid analysis. The analytical details are shown in the Table II.

**Acknowledgment.** We thank the Council of Scientific and Industrial Research, New Delhi, for awarding a senior research fellowship to A.A. The microanalyses provided by the Regional Sophisticated Instrumentation Centre, Lucknow, are gratefully acknowledged.

**Registry No.** BOC-Ala-OH, 15761-38-3; BOC-Leu-OH, 13139-15-6; BOC-Val-OH, 13734-41-3; BOC-Asp(OBzl)-OH, 7536-58-5; BOC-Gly-OH, 4530-20-5; BOC-Pro-Gly-OH, 51785-82-1; BOC-Ala-Gly-Val-OH, 56133-97-2; BOC-Leu-Ala-Gly-Val-OH, 61165-83-1; $O_2NC_6H_4$-*o*-COCl, 610-14-0; styrene–divinylbenzene copolymer, 9003-70-7.

# Synthesis of (4*S*)- and (4*R*)-Methyl 2-Amino-1-pyrroline-5-carboxylates and Their Application to the Preparation of (4*S*)-(+)- and (4*R*)-(-)-Dihydrokikumycin B

Moses Lee and J. William Lown*

*Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2, Canada*

*Received April 7, 1987*

The syntheses of the two enantiomeric forms of dihydrokikumycin B [(4*S*)-(+)-**7a** and (4*R*)-(-)-**7b**] with enantiomeric excess of 80 ± 4% are described. For this purpose, the 2-amino-1-pyrroline system and the pyrrole unit were prepared separately, and then subsequent coupling of these two groups afforded the carbon framework of **7a** and **7b**. Both antipodal forms of the 2-amino-1-pyrroline synthon, (4*S*)-**11a** and (4*R*)-**11b**, were prepared from the corresponding (*S*)- and (*R*)-pyroglutamic acids. Both enantiomers of the 2-pyrrolidone analogues [(4*S*)-(+)-**20a** and (4*R*)-(-)-**20b**] of dihydrokikumycin B were also synthesized. These optically active compounds bind to duplex native DNA with the following constants: (+)-**7a**, 1.24 ± 0.1; (-)-**7b**, 1.74 ± 0.1; (+)-**20a**, 0.35 ± 0.1; (-)-**20b**, 0.14 ± 0.1 × $10^6$ $M^{-1}$.

The modest family of naturally occurring oligopeptide antibiotics includes kikumycin B (**1**),[1] anthelvencin A (**2**),[2] distamycin (**3**),[3] netropsin (**4**),[4] amidomycin (**5**),[5] and noformycin (**6**)[6] (Figure 1). These agents have generated considerable interest on the part of synthetic chemists and pharmacists, owing to their broad spectrum of biological properties, such as antiviral, antibacterial, and anticancer activities.[3b,7] The biological activities of netropsin and distamycin appear to arise, in part, from their unique ATTT and ATTTT sequence specificity and minor groove-selective binding to DNA.[8] Physical studies, including X-ray analysis of a complex of netropsin with the symmetrical dodecamer $d(CGCGAATTCGCG)_2$,[8b] $^1H$ NMR investigation,[9] and CD studies,[7,9c] have provided structural details on the nature of the interactions between drug and receptor that contribute to the marked specif-

(1) (a) Takaishi, T.; Sugawara, Y.; Suzuki, M. *Tetrahedron Lett.* **1972**, 1873. (b) Kikuchi, M.; Kumagai, K.; Ishida, N.; Ito, Y.; Yamaguchi, T.; Furumai, T.; Okuda, T. *J. Antibiot., Ser. A* **1965**, *18*, 243.

(2) Probst, G. W.; Hoehn, M. M.; Woods, B. L. *Antimicrob. Agents Chemother. (1961–1970)* **1965**, 789.

(3) (a) Arcamone, F.; Orezzi, P. G.; Barbieri, W.; Nicolella, V.; Penco, S. *Gazz. Chim. Ital.* **1967**, *97*, 1097. (b) Hahn, F. E. In *Antibiotics III. Mechanism of Action of Antimicrobial and Antitumor Agents*; Corcoran, J. W., Hahn, F. E., Eds.; Springer-Verlag: New York, 1975; p 79.

(4) Julia, M.; Préau-Joseph, N. *Bull. Soc. Chim. Fr.* **1967**, 4348.

(5) Nakamura, S.; Karasawa, K.; Yonehara, H.; Tanaka, N.; Umezawa, H. *J. Antibiot., Ser. A* **1961**, *14*, 103.

(6) Diana, G. D. *J. Med. Chem.* **1973**, *16*, 857.

(7) Zimmer, C.; Wähnert, U. *Prog. Biophys. Mol. Biol.* **1986**, *47*, 31.

(8) (a) Zimmer, C. *Prog. Nucleic Acid Res. Mol. Biol.* **1975**, *15*, 285. (b) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1376.

(9) (a) Patel, D. J.; Kozlowski, S. A.; Rice, J. A.; Broka, C.; Itakura, K. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 7281. (b) Patel, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 6424. (c) Gupta, G.; Sarma, M. H.; Sarma, R. H. *J. Biomol. Struct. Dyn.* **1984**, *1*, 1457.

0022-3263/87/1952-5717$01.50/0 

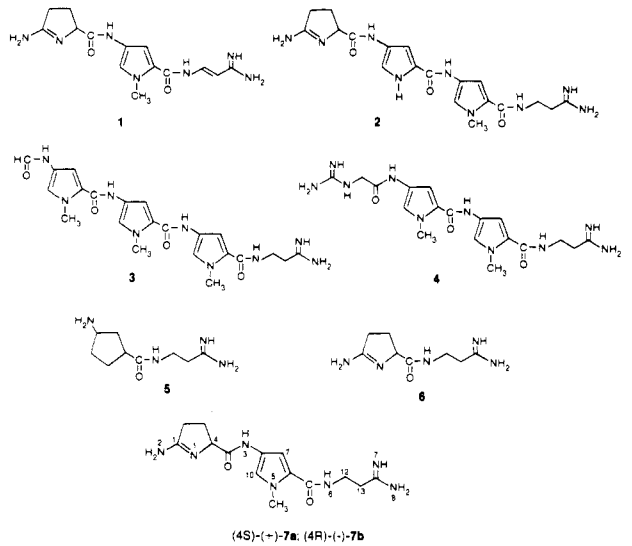



**Figure 1.** Kikumycin B (1), anthelvencin A (2), distamycin (3), netropsin (4), amidomycin (5), noformycin (6), and dihydrokikumycin B [(4*S*)-**7a**, (4*R*)-**7b**].

icity. Recently, the prediction[8b,10] has been confirmed that replacement of the pyrrole unit(s) in **3** and **4** with imidazole rings affords compounds that show acceptance of G·C sites.[10] This implies the formation of new hydrogen bonds between G-C(2)$NH_2$ in the minor groove and the additional $N_3$ imidazole nitrogen.[10] The firm and site-specific binding between these information-reading oligopeptides or "lexitropsins" and DNA is the net result of specific hydrogen bonding, electrostatic attraction, and van der Waals interactions.[8]

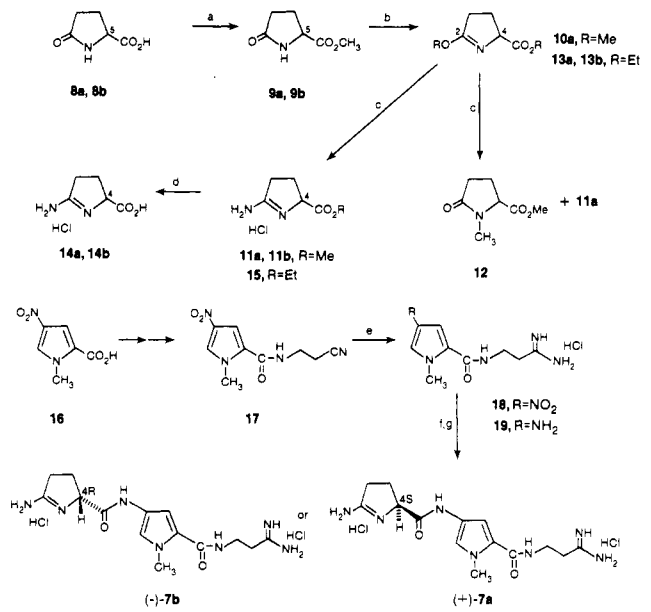
Thus far, none of the lexitropsins studied possesses a chiral center. Our continuing interest in molecular recognition has led us to explore the synthesis of related lexitropsins that are optically active. The synthesis of chiral dihydrokikumycin B, (+)-**7a** and (−)-**7b**, the former compound being the hydrogenation product of 1,[1] were selected for our synthetic study for the following reasons. First, the modes of interaction of the 2-amino-2-pyrroline moiety in kikumycin B (1) and in **2** with DNA are unknown. Second, both antipodal forms of the starting material (*S*)-(−)- and (*R*)-(+)-pyroglutamic acid (**8a** and **8b**, respectively) are readily available, and thus would provide an opportunity to prepare both enantiomers of dihydrokikumycin B, **7a** and **7b**. This would then allow us to study the effects of changes at the chiral center at C(4) of **7a** and **7b** on their ability to bind to DNA. To date, of all the known lexitropsins that have a 2-amino-1-pyrroline moiety, only noformycin **6** has been synthesized.[6]

In this paper, we report an efficient synthesis of the 2-amino-1-pyrroline synthon and subsequent coupling to an aminopyrrole unit, leading to the convergent syntheses of (+)- and (−)-dihydrokikumycin B (**7a** and **7b**, respectively). These compounds permit an assessment of the role of the chiral center in these optically active lexitropsins on binding to DNA.

## Results and Discussion

**Synthetic Strategy.** The structures of the antibiotic kikumycin B (1) and its dihydro derivative **7a** were established in 1972,[1] and until now, there have been no reported syntheses of these compounds. Recently, we have reported new and efficient syntheses of distamycin (**3**) and

**Scheme I**[i,ii]




[i] The letter a designates the 5*S* and 4*S* configuration, and b represents the 5*R* and 4*R* configuration. [ii] Reaction conditions: (a) MeOH, $SOCl_2$, DMF, room temperature, 17 h; (b) $(Et_3O)BF_4$, $CH_2Cl_2$, room temperature, 44 h; (c) $NH_4Cl$, MeOH, heat, 5 h; (d) 10% HCl, 50 °C, 2.5 h; (e) HCl in dry EtOH, then dry $NH_3$, EtOH; (f) $H_2$, Pd/C, MeOH, room temperature; (g) **14a**, DCC, DMAP, DMF, room temperature, 16 h.

netropsin (**4**)[11] that are sufficiently adaptable for the syntheses of (+)-**7a** and (−)-**7b**. The approach employed for the syntheses of both enantiomers of dihydrokikumycin B, as depicted in Scheme I, requires the preparation of the 2-amino-1-pyrroline moiety and the pyrrole unit separately, and then coupling of these two groups should afford the carbon skeleton of **7a** and **7b**.

The chiral starting materials for the direct enantioselective syntheses of **7a** and **7b** are the methyl esters **9a** and **9b**, which could be prepared readily from (*S*)-(−)-**8a** and (*R*)-(+)-**8b**, respectively.[12] Acid **8b** can be readily synthesized by refluxing (*R*)-(−)-glutamic acid in water.[13] The first step in our approach to prepare **7a** and **7b** is the transformation of the 2-pyrrolidone moiety in **9a** and **9b**, respectively, into the corresponding 2-amino-1-pyrroline moiety by way of the intermediacy of an imidate ester. Both **7a** and **7b** are prepared according to the procedures given in Scheme I; however, only the synthesis of **7a** will be described in detail. According to a recently published procedure, treatment of **9a** with dimethyl sulfate gave the methyl imidate ester **10a**.[14] However, subsequent treatment of **10a** with ammonium chloride in refluxing methanol gave the desired product **11a** in low yield (20%). The major product obtained is the *N*-methylpyrrolidone **12** (40%), which is presumably formed from tautomerization of the methyl group in **10a**. We anticipated that the ethyl imidate ester would be more stable toward rearrangement.[15] Accordingly, treatment of **9a** with Meerwein salt

(10) (a) Lown, J. W.; Krowicki, K.; Bhat, U. G.; Skorobogaty, A.; Ward, B.; Dabrowiak, J. C. *Biochemistry* **1986**, *25*, 7408. (b) Lown, J. W.; Krowicki, K.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1986**, *29*, 1210.

(11) Lown, J. W.; Krowicki, K. *J. Org. Chem.* **1985**, *50*, 3774.

(12) Drauz, K.; Kleeman, A.; Martens, J.; Scherberich, P. *J. Org. Chem.* **1986**, *51*, 3494.

(13) Hardy, P. M. *Synthesis* **1978**, 290.

(14) Nagasaka, T.; Tsukada, A.; Hamaguchi, F. *Heterocycles* **1986**, *24*, 2015.

(15) (a) Oishi, T.; Nagai, M.; Onuma, T.; Moriyama, H.; Tsutae, K.; Ochiai, M.; Ban, Y. *Chem. Pharm. Bull.* **1969**, *17*, 2306. (b) Bertele, E.; Boos, H.; Dunitz, J. D.; Elsinger, F.; Eschenmoser, A.; Felner, I.; Gribi, H. P.; Gschwend, H.; Meyer, E. F.; Pesaro, M.; Scheffold, R. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 490.

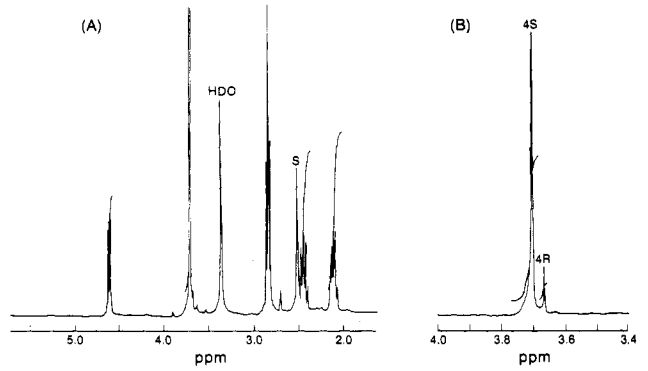


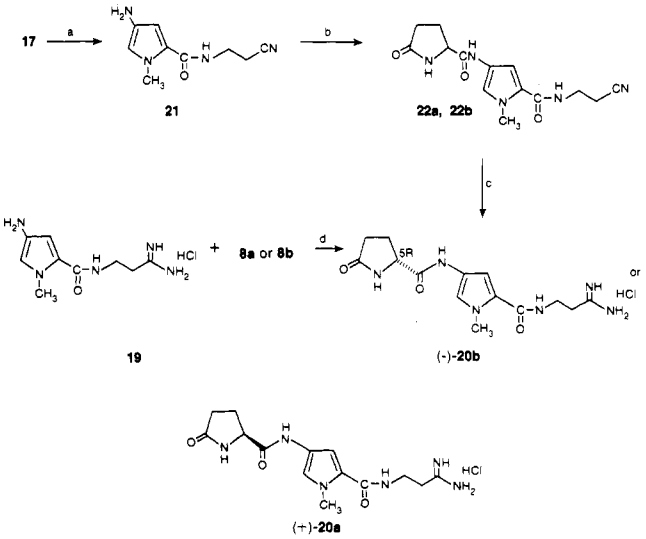

**Figure 2.** (a) 400-MHz $^1H$ NMR spectrum of ester **11a** in DMSO-$d_6$. (B) The $^1H$ NMR spectrum of **11a** in 1:1 $CD_3CN$/DMSO-$d_6$ with about 0.2 equiv of the chiral shift reagent.

(triethyloxonium tetrafluoroborate)[16] gave the desired ethyl imidate ester **13a** in 95% yield. The presence of the imidate ester moiety in **13a** is confirmed by the imine stretch at 1643 $cm^{-1}$ in the IR spectrum. Ester **13a** was converted with ammonium chloride in refluxing methanol to afford the intermediate **11a** in almost quantitative yield. The composition of **11a** is established by the appearance of the base peak at $m/z$ 143 for the M – Cl fragment. In addition, the presence of the amidine group in **11a** is confirmed by the appearance of an amidine stretch at 1682 $cm^{-1}$ in the IR spectrum and three exchangeable protons at $\delta$ 9.88, 9.55, and 9.21 ppm in the $^1H$ NMR spectrum. $^1H$ NMR analysis of ester **11a** in the presence of a chiral shift reagent (tris[3-[(trifluoromethyl)hydroxymethylene]-(+)-camphorato]europium(III)) as illustrated in Figure 2 shows about 5% racemization for the overall transformation from **8a**. Next, the ester moiety in **11a** was hydrolyzed in aqueous hydrochloric acid (10%) to give acid **14a** in high yield.[17]

The pyrrole unit required for the synthesis of **7a** can be readily synthesized from 1-methyl-4-nitropyrrole-2-carboxylic acid (**16**).[11] Nitration of **16** followed by coupling with $\beta$-aminoethyl cyanide gave nitrile **17**.[11] Pinner reaction of **17** (HCl in ethanol and then ammonia)[18] gave amidine hydrochloride **18** in high yield.[19] The nitro group in **18** was reduced catalytically to give amine **19**, which when allowed to react with acid **14a** in the presence of dicyclohexylcarbodiimide (DCC) gave (+)-dihydrokikumycin B **7a** in a moderate yield of 55%. The optical purity of (4S)-(+)-**7a** determined by $^1H$ NMR in the presence of a chiral shift reagent is 80 ± 4% enantiomeric excess. These conversions allow the 4S configuration to be assigned to the natural products (+)-**1** and (+)-**7a**.

Next we were also interested in comparing the DNA binding ability of the 2-amino-1-pyrroline moiety in **7a,b** to their pyrrolidone analogues **20a,b**. Our initial attempt to prepare **20a,b** was based on the approach to the synthesis of noformycin by Diana,[6] and it is depicted in Scheme II. Hydrogenation of the nitro compound **17** over palladium on charcoal gave the unstable amine intermediate **21**, which was directly coupled with acid **8a** to give pyrrolidone **22** in good yield (71%). However, Pinner reaction on **22** gave a complex product mixture, and analysis of the $^1H$ NMR spectrum of the crude material

**Scheme II**[i,ii]




[i] The latter a designates the 5S and 4S configuration, and b represents the 5R and 4R configuration. [ii] Reaction conditions: (a) $H_2$, Pd/C, MeOH, room temperature; (b) **8a** or **8b**, DCC, DMAP, DMF, room temperature, 16 h; (c) HCl in dry EtOH, then dry $NH_3$, EtOH; (d) DCC, DMAP, DMF, room temperature, 16 h.

showed only a small amount of the desired product **20a** (judged by the intensity of the low-field amide N*H* signal at 10.1 ppm). An alternative route to prepare **20a** would be to condense amine **19** with acid **8a**, since this method would avoid the problematic Pinner reaction step as previously encountered with **22**. Accordingly, reaction of amine **19** with **8a** in the presence of DCC gave (+)-**20a** in moderate yield (50%).

**Binding of Oligopeptides to DNA.** Both enantiomers of dihydrokikumycin B, (+)-**7a** and (-)-**7b**, and their 2-pyrrolidone analogues, (+)-**20a** and (-)-**20b**, all bind to duplex native DNA, and their binding constants to calf thymus DNA are as follows: (+)-**7a**, 1.24; (-)-**7b**, 1.74; (+)-**20a**, 0.35; (-)-**20b**, 0.14 × $10^6$ $M^{-1}$. The uncertaintity for the binding constants is ±0.1 × $10^6$ $M^{-1}$. Under these conditions, the binding constant for netropsin is 1.87 × $10^6$ $M^{-1}$.[20] The binding, while strong for all compounds, appears to be sensitive to the absolute configuration of the oligopeptides, since (4R)-(-)-**7b** and (5S)-(+)-**20a** bind more strongly to DNA than their respective enantiomers (4S)-(+)-**7a** and (5R)-(-)-**20b**. This finding provides an additional component in the design of sequence specific oligopeptide agents to further delineate the molecular recognition process in gene control. The incorporation of chiral end groups into specific oligopeptide vectors and the consequent sequence specificity and binding at individual sites deduced from quantitative DNase and MPE footprinting together with the effects of chirality on biological properties will be reported in due course.

## Conclusions

An efficient synthetic route to oligopeptides bearing a chiral 2-amino-1-pyrroline moiety (such as dihydrokikumycin B, (+)-**7a** and (-)-**7b** has been developed. This paper also shows that the binding ability of lexitropsins, related to netropsin (**4**), to DNA is sensitive to the chirality of the oligopeptides. This finding provides an additional chiral probe for the design of sequence specific DNA binding ligands for possible application as gene control

(16) (a) Meerwein, H. *Org. Synth.* **1966**, *46*, 113. (b) Paquette, L. A. *J. Am. Chem. Soc.* **1964**, *86*, 4096.

(17) It is essential that dry low-boiling alcohol solvents (e.g. ethanol) should not be used to coevaporate the trace amount of water during the workup stage for acid **14a**. Acid **14a** can undergo spontaneous esterification with ethanol to produce the ethyl ester **15** in quantitative yield.

(18) Pinner, A.; Klein, F. *Ber. Dtsch. Chem. Ges.* **1877**, *10*, 1889.

(19) Arcamone, F.; Nicolella, V.; Penco, S.; Redaelli, S. *Gazz. Chim. Ital.* **1969**, *99*, 632.

(20) (a) Morgan, A. R.; Lee, J. S.; Pulleyblank, D. E.; Murray, N. L.; Evans, D. H. *Nucleic Acid Res.* **1979**, *7*, 547. (b) Le Pecq, J.-B.; Paoletti, C. *J. Mol. Biol.* **1967**, *27*, 87.

agents, in addition to the replacement of pyrrole units with imidazole or other heterocycles as reported earlier.[10]

## Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The IR spectra were recorded on a Nicolet 7199 FT spectrophotometer, and only the principal bands are reported. The $^1H$ NMR spectra were recorded on Bruker WH-200 and WH-400 spectrometers. FAB (fast atom bombardment) mass spectra were determined on an Associated Electrical Industries (AEI) MS-9 and MS-50 focusing high-resolution mass spectrometer. Kieselgel 60 (230–400 mesh) of E. Merck was used for flash chromatography, and precoated silica gel 60F-254 sheets of E. Merck were used for TLC, with the solvent system indicated in the procedure. TLC plates were visualized by using UV light or 2.5% phosphomolybdic acid in methanol with heating.

All compounds obtained commercially were used without further purification unless otherwise stated. Ethanol and methanol were freshly distilled from magnesium turnings, tetrahydrofuran was distilled from sodium/benzophenone under an atmosphere of dry argon, ether was dried over sodium, methylene chloride was distilled from phosphorus pentoxide and stored over molecular sieves (3A), triethylamine was treated with potassium hydroxide and then distilled from barium oxide and stored over molecular sieves 3A, and dimethylformamide was distilled from barium oxide and stored over molecular sieves (3A).

**(4*S*)-2-Ethoxy-5-(methoxycarbonyl)-1-pyrroline (13a).** To a stirring solution of ester **9a** (5.0 g, 35.0 mmol) in dry methylene chloride (50.0 mL) was added a solution of triethyloxonium tetrafluoroborate (50.0 mL, 50.0 mmol, 1.0 M stock solution) in $CH_2Cl_2$. The reaction mixture was stirred under argon at room temperature for 44 h, at which time TLC analysis showed complete disappearance of the starting material. A solution of saturated potassium bicarbonate (25.0 mL) was added, andd when the effervescence had subsided, the $CH_2Cl_2$ layer was removed. The aqueous phase was extracted with $CH_2Cl_2$ (40 mL), and the combined organic layers were dried ($MgSO_4$). Concentration of the organic extracts gave **13a** as a clear oil (5.73 g, 95% yield), which was pure by examination on TLC and $^1H$ NMR: TLC (10% MeOH/$CHCl_3$) $R_f$ 0.93; IR ($CHCl_3$) 1743, 1643, 1376, 1337, 1202, 1177, 1031 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 4.50 (dd, 5.7, 6.9, H4), 4.24 (q, 6.8, $CH_2O$), 3.73 (s, $OCH_3$), 2.0–2.6 (m, H2 and H3), 1.28 (t, 6.8, $CH_3C$); exact MS, $m/z$ (relative intensity) for $C_8H_{13}NO_3$ 171.0896 ($M^+$, 3.6), $C_6H_{10}NO$ 112.0763 (31), $C_4H_6NO$ 84.0483 (100).

**Methyl (4*S*)-(−)-2-Amino-1-pyrroline-5-carboxylate Hydrochloride (11a).** A stirring solution of imidate ester **13a** (5.73 g, 33.0 mmol) and anhydrous ammonium chloride (1.90 g, 35.0 mmol) in dry methanol (60.0 mL) under argon atmosphere was heated to reflux for 5 h. Removal of the solvent under reduced pressure gave an essentially pure (based on TLC and $^1H$ NMR analyses) white solid, which was washed with $CCl_4$ than dried in vacuo at 80 °C for 2 h to afford **11a** (5.97 g, 95% yield). A small sample was recrystallized from $CH_2Cl_2$/hexane to give **11a**: mp 169–171 °C; TLC (20% MeOH/$CHCl_3$ and a few drops of acetic acid) $R_f$ 0.43; $[\alpha]^{22}_D$ −1.6 (*c* 0.018 g/mL, $H_2O$); IR (Nujol) 3301, 1740, 1682, 1437, 1274, 1232 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 9.88, 9.55, and 9.21 (3 s, 1 H each, amidine H's), 4.60 (dd, 4.7, 9.2, H4), 3.70 (s, $OCH_3$), 2.83 (t, 9.8, H2), 2.40 (m, H3a), 2.07 (m, H3b); $^{13}C$ NMR (DMSO-$d_6$) $\delta$ 171.2 (C=O), 113.7 (C1), 60.0 ($OCH_3$), 52.4 (C4), 29.4 (C2), 24.6 (C3); FAB-MS (glycerol), $m/z$ (relative intensity) 499 (3M − HCl, 0.3), 285 (2M − H − 2Cl, 11.7), 143 (M − Cl, 100). Anal. Calcd for $C_6H_{11}N_2O_2Cl$ (178.45): C, 40.4; H, 6.2; N, 15.7. Found: C, 40.3; H, 6.2; N, 16.0.

**(4*S*)-(−)-2-Amino-1-pyrroline-5-carboxylic Acid Hydrochloride (14a).** A solution of ester **11a** (1.00 g, 5.63 mmol) in aqueous hydrochloric acid (10%) was stirred at about 50 °C for 2.5 h, at which time TLC analysis indicated complete hydrolysis of the ester. Concentration of the reaction mixture gave a white solid, which was coevaporated with dry $CH_2Cl_2$ (twice, 40 mL) and dry benzene (once). The product was collected and dried under high vacuum at about 80 °C to give acid **14a** (854.5 mg 93% yield), which was essentially pure by TLC and $^1H$ NMR analysis: mp 150–155 °C; TLC (10% MeOH/$CHCl_3$ and some HOAc) $R_f$ 0.26; $[\alpha]^{22}_D$ −8.2 (*c* 0.0095, $H_2O$); IR (Nujol) 3270, 1730, 1680, 1456, 1404, 1376 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 13.20 (s br, $CO_2H$), 9.87, 9.44, 9.10 (3 s, 3 amidine H's), 4.51 (dd, 4.6, 9.2, H4), 2.81 (t, 8.0, H2), 2.40 (m, H3a), 2.10 (m, H2b); FAB-MS (glycerol), $m/z$ (relative intensity) 385 (3M − 2H − 3Cl, 0.8), 331 (2M + 2H, 0.2), 257 (2M − H − 2Cl, 9.0), 143 (M − Cl, 100).

It is very important that dry low-boiling alcohol solvents (e.g. ethanol) should not be used to coevaporate acid **14a**, since it can undergo spontaneous reesterification to produce ester **15**: mp 105–109 °C; TLC (20% MeOH/$CHCl_3$ and some HOAc) $R_f$ 0.29; $[\alpha]^{22}_D$ −2.9 (*c* 0.0123, $H_2O$); IR (Nujol) 3269, 1737, 1694 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 9.87, 9.52, 9.18 (3 s, 3 amidine H's), 4.58 (dd, 4.8, 9.1, H4), 4.16 (q, 7.3, $OCH_2$), 2.83 (t, 7.6, H2), 2.44 (m, H3a), 2.10 (m, H3b), 1.22 (t, 7.3, $CCH_3$); FAB-MS (glycerol), $m/z$ (relative intensity) 349 (2M − Cl, 0.7), 313 (2M − H − 2Cl, 7.2), 157 (M − Cl, 100). Anal. Calcd for $C_7H_{13}N_2O_2Cl$ (192.45): C, 43.6; H, 6.8; N, 14.6. Found: C, 42.8; H, 6.6; N, 14.2.

**3-(1-Methyl-4-nitropyrrole-2-carboxamido)propionamidine Hydrochloride (18).** A suspension of nitrile **17** (307.2 mg, 1.38 mmol) in anhydrous ethanol (10.0 mL) was chilled (0 °C) and saturated with dry hydrogen chloride gas. The resulting solution was stirred at 0 °C for 1 h and then allowed to warm to ambient temperature. The reaction mixture was concentrated to give a solid residue, which was washed with dry ether (20.0 mL, twice). The residue was resuspended in dry ethanol (10.0 mL) and chilled (0 °C), and then dry ammonia was condensed into the reaction mixture. After 1 h at 0 °C, the solution was gradually warmed to room temperature. Concentration of the reaction mixture gave a solid, which was recrystallized from methanol to give amide **18** as as white powder (294.5 mg, 78% yield): mp 264–266 °C (lit.[19] mp 267–270 °C); TLC (10% MeOH/$CHCl_3$ and some HOAc) $R_f$ 0.35; IR (Nujol) 3239, 1651, 1459, 1314 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 9.04 (s br, 2 H), 8.76 (s br, 2 H), 8.75 (t, 6.0, 1 H), 8.14 (d, 1.9, 1 H), 7.54 (d, 1.9, 1 H), 3.90 (s, 3 H), 3.53 (q, 6.0, 2 H), 2.64 (t, 6.0, 2 H); FAB-MS, $m/z$ (relative intensity) 515 (2M − Cl, 0.2), 240 (M − Cl, 4.9). Anal. Calcd for $C_9H_{14}N_5O_3Cl$ (275.45): C, 39.2; H, 5.1; N, 25.4. Found: C, 38.7; H, 5.1; N, 24.5.

**(4*S*)-(+)-Dihydrokikumycin B (7a).** A solution of amidine **18** (207.0 mg, 0.75 mmol) in methanol (15.0 mL) was hydrogenated over 5% Pd on charcoal (98 mg) at room temperature and atmospheric pressure until TLC analysis indicated complete reduction of the starting material. The catalyst was removed by filtration. The filtrate was concentrated, and the residue was coevaporated with dry $CH_2Cl_2$ (30 mL, twice) to give amine **19** as a foamy white solid. Due to the instability of **19**, it was used directly in the following step. Acid **14a** (155.9 mg, 0.95 mmol) and 4-(dimethylamino)pyridine (DMAP; 12.0 mg, 0.98 mmol) was added to the amine. The mixture was dissolved in dry DMF (5.0 mL) and chilled (0 °C), followed by the addition of a solution of dicyclohexylcarbodiimide (DCC; 201.1 mg, 0.98 mmol) in dry DMF (3.0 mL). The reaction mixture was stirred under argon atmosphere at 0 °C for 15 min and at room temperature for 17 h. The precipitated urea was removed by filtration, and the filtrate was concentrated in high vacuum to give an oily residue. This residue was purified by flash chromatography (80:25:5, MeOH/$CHCl_3$/HOAc) to give a solid material, which was recrystallized from MeOH/acetone to give **7a** (162 mg, 55% yield): mp 212–215 °C; TLC (80:15:5 MeOH/$CHCl_3$/HOAc) $R_f$ 0.25; $[\alpha]^{22}_D$ +5.3° (*c* 0.0102, MeOH), $[\alpha]^{24}_D$ +13.2° (*c* 0.0053, $H_2O$) [lit.[1] $[\alpha]^{22}_D$ +15° ($H_2O$)]; UV ($H_2O$) 243 (3.95), 276 (3.86) [lit.[1] ($H_2O$) 237 (4.02), 278 (3.92) as the sulfate]; IR (Nujol) 3239, 1692, 1640, 1460, 1369 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 10.38 (s, NH3), 8.52 (s br, 7 amidine H's), 8.34 (t, 4.9, NH5), 7.17 (d, 1.7, H10), 6.82 (d, 1.7, H7), 4.66 (dd, 4.4, 9.0, H4), 3.79 (s, $NCH_3$), 3.50 (q, 5.0, H12), 2.81 (t, 6.8, H2), 2.69 (m, H3a), 2.61 (t, 5.0, H13), 2.06 (m, H3b), and after $D_2O$ exchange, 7.11 (d, 1.7, H10), 6.72 (d, 1.7, H7), 4.47 (dd, 4.8, 8.1, H4), 3.73 (s, $NCH_3$), 3.49 (t, 6.3, H12), 2.82 (t, 8.0, H13), 2.57 (t, 6.3, H2), 2.46 (m, H3a), 2.00 (m, H3b); FAB-MS, $m/z$ (relative intensity) 356 (M − Cl, 2.0), 355 (M − HCl, 3.0), 320 (M − H − 2Cl, 14.1), 287 (M − $2NH_4Cl$, 3.0).

**(5*S*)-(+)-3-[1-Methyl-4-(2-pyrrolidone-2-carboxamido)-pyrrole-2-carboxamido]propionamidine Hydrochloride (20a).** A mixture of amine **19** (prepared from 310.5 mg, 1.13 mmol of **17** following the procedure for **7a**), (*S*)-(−)-pyroglutamic acid **8a** (180.0 mg, 1.39 mmol), and DMAP (29.4 mg, 0.24 mmol) was dissolved in dry DMF (4.0 mL) and then chilled (0 °C). A solution of DCC (301.2 mg, 1.46 mmol) in dry DMF (2.0 mL) was introduced. After 15 min at 0 °C, the reaction mixture was stirred

overnight under an atmosphere of argon. Removal of the urea and concentration of the filtrate gave a residue which was purified by flash chromatography (60:35:5 MeOH/$CHCl_3$/HOAc) and then crystallization from MeOH/acetone to afford **20a** (201.0 mg, 50% yield): mp 170–174 °C; TLC (20% MeOH/$CHCl_3$ and some HOAc) $R_f$ 0.26; $[\alpha]^{22}_D$ +4.7 (*c* 0.0143, MeOH); IR (Nujol) 3255, 1686, 1646, 1462 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 10.09 (s, NH), 9.04 (s br, 2 H, amidine), 8.75 (s, br, 2 H, amidine), 8.31 (t, 5.9, NH), 7.90 (s, NH), 7.16 (d, 1.4, 1 H), 6.81 (d, 1.4, 1 H), 4.14 (dd, 3.9, 7.3, 1 H), 3.79 (s, $NCH_3$), 3.48 (q, 5.9, 2 H), 2.59 (t, 5.9, 2 H), 2.41–1.85 (m, 4 H); FAB-MS, *m/z* (relative intensity) 320 (M – Cl, 6.2). Anal. Calcd for $C_{14}H_{21}N_6O_3Cl\cdot H_2O$ (374.45): C, 44.9; H, 6.1. Found: C, 45.5; H, 6.1.

**(5*S*)-3-[1-Methyl-4-(2-pyrrolidone-5-carboxamido)-pyrrole-2-carboxamido]propionitrile (22a).** A suspension of nitrile **17** (943.7 mg, 4.25 mmol) in MeOH (30.0 mL) was hydrogenated over 5% Palladium on charcoal (404 mg) at room temperature and atmospheric pressure. Removal of the catalyst and concentration of the filtrate gave an oily residue, which was coevaporated with dry $CH_2Cl_2$ (twice, 40 mL) to give amine **21** as a foamy off-white material. Owing to the instability of the amine, it was used directly in the following reaction. A mixture of acid **8a** (884.0 mg, 6.85 mmol), DMAP (78.0 mg, 0.64 mmol), and amine **21** was dissolved in dry DMF (15.0 mL) and chilled (0 °C). To this solution was added DCC (1.36 g, 6.50 mmol) in dry DMF (8.0 mL). After 15 min at 0 °C, the reaction mixture was stirred at room temperature overnight. Removal of the urea and the solvent gave a solid residue, which was recrystallized from MeOH to afford **22a** as a white powder (915.1 mg, 71% yield): mp 245–246 °C; TLC (10% MeOH/$CHCl_3$) $R_f$ 0.35; IR (Nujol) 3263, 2318, 1692, 1673, 1655, 1533, 1464, 1377 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 10.00 (s, 1 H), 8.36 (t, 6.2, 1 H), 7.87 (s, 1 H), 7.16 (d, 1.5, 1 H), 6.79 (d, 1.5, 1 H), 4.11 (dd, 4.2, 8.0, 1 H), 3.79 (s, 3 H), 3.90 (q, 6.2, 2 H), 2.72 (t, 6.2, 2 H), 2.29 (m, 1 H), 2.15 (m, 2 H), 1.92 (m, 1 H); exact MS, *m/z* (relative intensity) for $C_{14}H_{17}N_5O_3$ 303.1334 (M, 25.3), for $C_9H_{12}N_{14}O_2$ 192.1014 (M – pyroglutamoyl group, 55.9).

***R* Stereoisomers.** Compounds (4*R*)-(+)-**11b**, (4*R*)-(+)-**14b**, (4*R*)-(–)-**7b**, and (5*R*)-(–)-**20b** all gave IR, MS, and $^1H$ NMR data similar to those of the corresponding *S* isomers. The specific rotations, $[\alpha]^{22}_D$, are +2.0° (*c* 0.0051, MeOH), +7.7° (*c* 0.0044, $H_2O$), –6.3° (*c* 0.0050, MeOH), and –4.6° (*c* 0.0142, MeOH), respectively.

**Determination of DNA Binding Constants.** The relative binding constants were estimated by displacement of intercalative binding of ethidium to calf thymus DNA and employment of a value of $K_{assoc} = 1 \times 10^6$ $M^{-1}$ at pH 7.0, 37 °C, and 40 mM NaCl for ethidium bound to calf thymus DNA.[20] It was determined that none of the oligopeptides interferes with the fluorescence measurements, which were performed on a Turner 430 spectrofluorometer. The procedure, which involves following the displacement of the ethidium upon titrating in the drugs and determining the concentration of drug required to displace 50% of the ethidium, follows that of Morgan and co-workers[20a] and gives relative rather than absolute values for binding constants. Higher concentrations of lexitropsins displace all the ethidium from the DNA.

**Acknowledgment.** This investigation was supported by grants (to J.W.L.) from the Biotechnology Strategic Grants Programme of the Natural Sciences and Engineering Research Council of Canada and by the National Cancer Institute of Canada.

**Registry No.** **7a**, 111136-83-5; (4*R*)-(–)-**7b**, 111136-84-6; **8a**, 98-79-3; **9a**, 4931-66-2; **11a**, 110971-80-7; (4*R*)-(+)-**11b**, 110971-85-2; **13a**, 65571-69-9; **14a**, 110971-81-8; (4*R*)-(+)-**14b**, 110971-86-3; **15**, 110971-82-9; **17**, 3185-95-3; **18**, 24064-13-9; **19**, 78395-16-1; **20a**, 110971-83-0; (5*R*)-(–)-**20b**, 110971-87-4; **21**, 97950-77-1; **22a**, 110971-84-1.

# Synthesis of 1,2,5,6-Tetrahydrophosphorin 1-Oxides and 1,2-Dihydrophosphorin 1-Oxides from 2,5-Dihydro-1*H*-phosphole 1-Oxide–Dichlorocarbene Adducts

György Keglevich, Gábor Tóth, Imre Petneházy, Péter Miklós, and László Töke*

*Organic Chemical Technology Department, Technical University of Budapest, H-1521 Budapest, Hungary*

*Received April 8, 1987*

Reaction of 6,6-dichloro-1-methyl-3-phosphabicyclo[3.1.0]hexane 1-oxides **1** with silver nitrate in alcohols or in water leads to the regioisomers of alkoxy- or hydroxy-1,2,3,6-tetrahydrophosphorin 1-oxides **2A** and **2B** or **3A** and **3B**. Each isomer consists of diastereoisomers. Water elimination from the hydroxy derivatives **3** results in the formation of the regioisomers of 1,2-dihydrophosphorin 1-oxides **4A** and **4B**. Constitution and stereostructure of the compounds has been elucidated by $^1H$, $^{13}C$, and $^{31}P$ NMR spectroscopy.

Although the enlargement of five- and six-membered unsaturated rings by the addition of dihalocarbene and subsequent transformations is a well-established method,[1-3] there have been no reports on this kind of ring expansion for phosphorus-containing heterocycles. Recently we reported on the dichlorocarbene addition to 2,5-dihydro-1*H*-phosphole 1-oxides and on the spontaneous transformation of certain adducts to 1,2-dihydrophosphorin 1-oxides.[4] In this paper the synthesis of dihydrophosphorins through tetrahydrophosphorins starting from stable 1*H*-phosphole–dichlorocarbene adducts is discussed.

## Results and Discussion

Dihalocarbene adducts can be enlarged in several ways; one of them involves the use of silver salts.[1,3] Reaction of

(1) Skell, P. S.; Sandler, S. R. *J. Am. Chem. Soc.* **1958**, *80*, 2024.
(2) Parham, W. E.; Reiff, H. E.; Swartzentruber, P. *J. Am. Chem. Soc.* **1956**, *78*, 1437. Winberg, H. E. *J. Org. Chem.* **1959**, *24*, 264. Cromarty, A.; Haque, K. E.; Proctor, G. R. *J. Chem. Soc. C* **1971**, 3536. Perchonock, C. D.; Lantos, I.; Finkelstein, J. A.; Holden, K. G. *J. Org. Chem.* **1980**, *45*, 1950.
(3) Lantos, I.; Bhattacharjee, D.; Eggleston, D. S. *J. Org. Chem.* **1986**, *51*, 4147.
(4) Keglevich, Gy.; Petneházy, I.; Miklós, P.; Almásy, A.; Tóth, G.; Töke, L.; Quin, L. D. *J. Org. Chem.* in press.

0022-3263/87/1952-5721$01.50/0 