Practical Synthesis of Active Esters of 4-Alkoxycarbonylamino-3-methoxypyrrole-2-carboxylic Acid

Kesavaram Narkunan, Marco A. Ciufolini*

Université Claude Bernard Lyon 1 and Ecole Supérieure de Chimie, Physique, Electronique de Lyon, 43, Boulevard du 11 Novembre 1918, 69622 Villeurbanne cedex, France

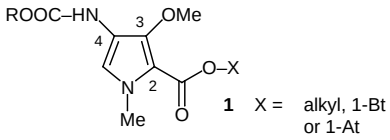
Fax +33(4)72432963; E-mail: ciufi@cpe.fr

Received 6 October 1999; revised 5 January 2000

Abstract: An efficient large scale synthesis of the HOAt active ester of 4-*tert*-butoxycarbonylamino-3-methoxypyrrole-2-carboxylic acid is reported.

Key words: distamycin, DNA, pyrroles, Curtius rearrangement, transesterifications, heterocycles

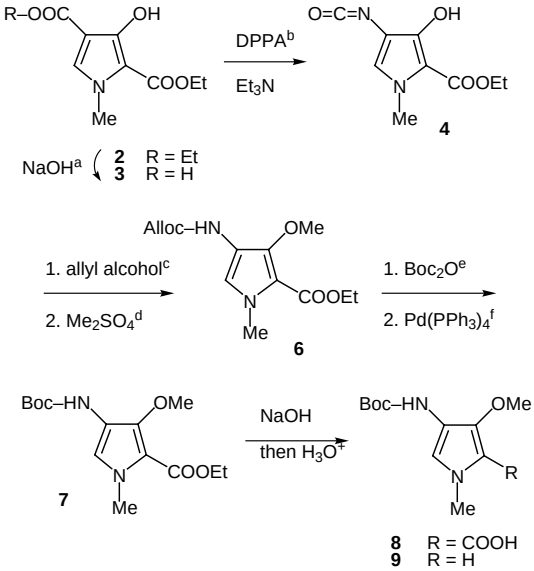
The possibility of achieving digital readout of DNA by chemical methods is becoming increasingly apparent thanks especially to the work of Dervan and collaborators.[1] These workers have shown that analogs of the antitumor agent, distamycin,[2] are capable of sequence-specific recognition of double-stranded DNA.[3] The assembly of such constructs requires quantities of various heterocyclic building blocks,[4] including active esters of deceptively simple pyrroles of general structure **1** (R = alkyl, X = 1-benzotriazolyl or 1-pyridotriazolyl).[5] The importance of **1** stems from its function as a recognition element for thymine. Thus, unit **1** is incorporated into a pyrrolic polyamide and the C-3 "phenolic" OH is ultimately liberated. This hydroxyl group, together with the C-4 amido NH function, is believed to establish a bifurcate H-bond to the carbonyl group of thymine.[5]



1 X = alkyl, 1-Bt or 1-At

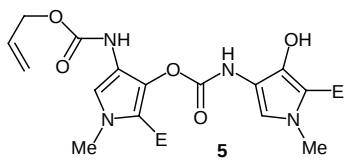
Whereas methods for the preparation of most of the foregoing building blocks are described in the primary literature,[4] the synthesis of compounds related to **1**, on scales of a few hundred milligrams, has been recorded only in a patent.[5,10] Ongoing research required quantities of an active ester of **1**, wherein the 4-amino group is blocked as an *N*-Boc derivative. Such an intermediate has been claimed in the above patent,[5] but details of its preparation are not provided.[10]

Our first attempts to produce the desired goal proceeded through modifications of the patented procedures, which evolve from diethyl ester **2**, readily available in 100 g batches through a published, fully reproducible method.[6] In our hands, modifications of public-domain protocols were entirely unsatisfactory for the synthesis of multigram lots of **1**. The presence of a free "phenolic" OH in **2** permits chemoselective base hydrolysis of the ester at position 4 to give acid **3**. The Yamada modification[7] of the Curtius rearrangement of **3** produces isocyanate **4**.[8] Attempted reaction of **4** with *t*-BuOH gave a complex mixture containing at best trace amounts of the desired Boc product, even when capture of the isocyanate with *t*-BuOH was attempted in the presence of catalysts such as CuCl.[9] In complete accord with Dervan,[5] more reactive allyl alcohol did combine with the isocyanate to produce the corresponding allyl carbamate, but only in 30−40% yield. A compound tentatively identified as **5** was also obtained from this reaction, raising questions about the viability of a free OH group during the Yamada step. Nonetheless, production of the desired *N*-Boc intermediate in gram amounts became possible as shown in Scheme 1. The allyl carbamate was *O*-methylated to furnish **6**, which reacted with Boc_2O to give a mixed Alloc-Boc imide. Selective Alloc cleavage under catalysis by Pd(0) then provided ester **7**. An even more troublesome difficulty emerged during hydrolysis of **7** in accord with the published procedure,[5] which calls for prolonged (3 days) heating of



Reagents and conditions: a) $EtOH/H_2O$, 100°C, 6 h, 75%; b) MeCN, 85°C, 2 h; c) 100°C, 17 h, 40%; d) K_2CO_3/acetone, reflux, 24 h, 85%; e) TEA/DMAP/THF, r.t., 16 h, 93%; f) dimedone/THF, r.t., 2 h, 80%

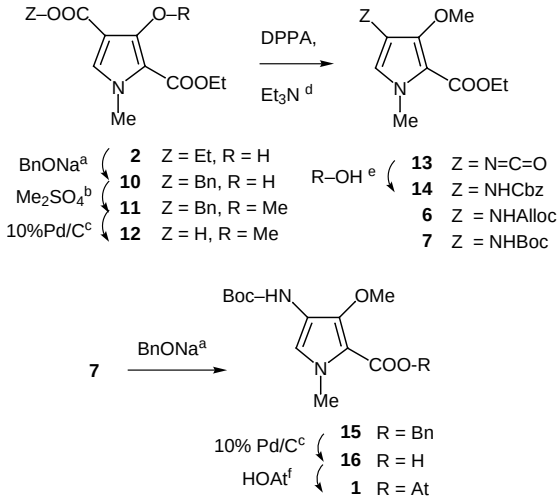
Scheme 1



5

the substrate at 100°C in strongly basic medium. We were never able to obtain acid **8** in greater than 30−35% yield, due to its very facile, and not unexpected, decarboxylation to pyrrole **9**. No simple solution to this problem could be found.

Our observations suggest that an expressed pyrrolic OH group is largely incompatible with the Curtius sequence, even though its presence is required in order to achieve chemoselective ester hydrolysis of **2**. Moreover, the vigorous conditions applied to saponification of the C-2 ester in **7** must be avoided. We thus researched a new protocol for the synthesis of **1** (R = *t*-Bu, X = 1-At) on large scale. A robust process was developed as shown in Scheme 2.



Reagents and conditions: a) BnOH, 100°C, 10 Torr, 4 h, 79% for **10**, 87% for **15**; b) K_2CO_3/acetone, reflux, 24 h, 88%; c) H_2, 50 bar, ~100%; d) MeCN, 85°C, 2 h; e) allyl alcohol (80%) or *t*-BuOH (70%) or BnOH (78%), 100 °C, 17 h; f) DCC/DMAP/CH_2Cl_2, r.t., 20 h, 90%

Scheme 2

Exposure of **2** to sodium benzyloxide induced chemoselective transesterification at the C-4 carboxy unit. The emerging **10** was *O*-methylated prior to hydrogenolysis of the benzyl group and intermediate acid **12** was subjected to Curtius rearrangement. The presumed isocyanate intermediate[8] was readily intercepted with allyl alcohol, *t*-BuOH or BnOH to furnish the corresponding carbamates **6**, **7** and **14** in 80, 70 and 78% yields, respectively. A second transesterification of **7** exchanged the C-2 ethyl ester with a benzyl ester, which was hydrogenolyzed at room temperature to give the sensitive acid **16** in quantitative yield.

Compound **16** was not extensively characterized, due to its propensity to undergo facile decarboxylation; rather, it was immediately condensed with, e.g., HOAt in the presence of DCC and 4-DMAP to furnish active ester **1** (R = At) in high yield. Material of sufficient purity (NMR, elemental analysis) for use in the synthesis of Dervan polyamides was obtained after a simple precipitation from a CH_2Cl_2 solution by addition of hexane.

In summary, a protocol for the synthesis of active esters of **1** on multigram scale is now available. The new procedure should be a welcome improvement over published routes to the target systems, on accounts of their importance in nucleic acid, genomics and medicinal chemistry research.

All sensitive reactions were carried out under argon. BnOH and allyl alcohol were stored over anhyd K_2CO_3 before use. THF was freshly distilled from Na/benzophenone. *t*-BuOH was freshly distilled from Na before use. MeCN, CH_2Cl_2 and TEA were freshly distilled from CaH_2. DPPA, dimethyl sulfate and K_2CO_3 (325 mesh) were used as received. Unless otherwise indicated, NMR spectra (δ, ppm, 300 MHz for ^1H; 75 MHz for ^{13}C; 25°C, coupling constants *J* in Hz) were recorded in $CDCl_3$ with TMS as internal standard; FTIR spectra (cm^{-1}) were obtained from thin films on NaCl plates (oils) or from KBr pellets (solids). Low and high resolution mass spectra (*m*/*z*) were obtained in EI mode. Microanalyses were carried out at the CNRS analytical center in Solaize (Lyon).

4-Benzyl 2-Ethyl 1-Methyl-3-hydroxy-1*H*-pyrrole-2,4-dicarboxylate (10)

Compound **2** (20.0 g, 83.0 mmol) was added to an ice-cold solution of sodium metal (2.7 g, 116.0 mmol) in benzyl alcohol (172.0 mL). The resulting mixture was stirred at r.t. for 30 min, then it was heated at 100°C under reduced pressure (10 Torr) for 4 h, in order to completely remove the liberated EtOH. Most of the benzyl alcohol was then distilled by lowering of the pressure to 1 Torr with continued heating at 100°C. The residue thus obtained was partitioned between Et_2O (100 mL) and H_2O (20 mL) and the mixture was taken to pH 5 with 4 N HCl. The layers were separated and the aqueous phase was extracted with Et_2O (2 × 20 mL). The combined extracts were washed with sat. aq $NaHCO_3$ (20 mL) and brine (20 mL), dried (Na_2SO_4), and evaporated. Recrystallization of the solid residue (cyclohexane) afforded 20.0 g (79%) of pure **10**; mp 88°C.

^1H NMR: δ = 1.35 (t, *J* = 7.35 Hz, 3 H), 3.75 (s, 3 H), 4.33 (q, *J* = 7.35 Hz, 2 H), 5.26 (s, 2 H), 7.07 (s, 1 H), 7.20−7.40 (m, 5 H), 8.73 (s, 1 H).

^{13}C NMR: δ = 14.41, 38.08, 60.26, 65.58, 101.95, 107.55, 128.00, 128.07, 128.49, 130.12, 136.18, 154.34, 161.92, 163.86.

IR (KBr): ν = 3127, 1719, 1645 cm^{-1}.

MS: *m*/*z* = 304, 303 (M^+), 195, 150, 123, 91.

HRMS: *m*/*z* calcd for $C_{16}H_{17}NO_5$ 303.1107, found 303.1106.

Anal. calcd for $C_{16}H_{17}NO_5$: C 63.36, H 5.65, N 4.62; found: C 63.58, H 5.54, N 4.77.

4-Benzyl 2-Ethyl 1-Methyl-3-methoxy-1*H*-pyrrole-2,4-dicarboxylate (11)

Dimethyl sulfate (6.7 mL, 71.2 mmol) was added dropwise to a solution of **10** (18.0 g, 59.3 mmol) in anhyd acetone (180.0 mL) containing suspended K_2CO_3 (325 mesh, 16.4 g, 118.7 mmol). The resulting mixture was refluxed for 24 h under argon. Concentration left a residue that was partitioned between Et_2O (100 mL) and H_2O (50 mL). The aqueous layer was extracted with Et_2O (2 × 20 mL) and the combined extracts were dried (Na_2SO_4) and evaporated.

Purification of the residue by flash chromatography (100 g of silica gel, 20% EtOAc/hexane) afforded 16.6 g (88%) of pure **11** as an oil.

^{1}H NMR: δ = 1.36 (t, *J* = 7.35 Hz, 3 H), 3.83 (s, 3 H), 3.86 (s, 3 H), 4.32 (q, *J* = 7.35 Hz, 2 H), 5.27 (s, 2 H), 7.21 (s, 1 H), 7.25−7.45 (m, 5 H).

^{13}C NMR: δ = 14.35, 38.47, 60.15, 62.91, 65.63, 107.41, 114.76, 128.06, 128.07, 128.54, 130.79, 136.47, 153.27, 160.76, 162.50.

IR (neat): ν = 2981, 1696, 1551 cm^{-1}.

MS: *m*/*z* = 318, 317 (M^+), 255, 210, 180, 150, 91.

HRMS: *m*/*z* calcd for $C_{17}H_{19}NO_5$ 317.1263, found 317.1266.

Ethyl 4-Carboxy-3-methoxy-1-methyl-1*H*-pyrrole-2-carboxylate (12)

A solution of **11** (15.0 g, 47.3 mmol) in dioxane (150.0 mL) containing 10% Pd/C (750.0 mg) was stirred under hydrogen pressure (50 bar) overnight. The catalyst was filtered off (cotton plug) and washed with more dioxane. Concentration of the combined filtrates afforded solid acid **12** (10.7 g, ~100%); mp 153°C (EtOH).

^{1}H NMR: δ = 1.36 (t, *J* = 7.35 Hz, 3 H), 3.84 (s, 3 H), 3.91 (s, 3 H), 4.32 (q, *J* = 7.35 Hz, 2 H), 7.27 (s, 1 H).

^{13}C NMR: δ = 14.33, 38.69, 60.31, 63.14, 106.72, 114.80, 131.43, 153.34, 160.57, 167.36.

IR (KBr): ν = 2984, 1684, 1556, 1524 cm^{-1}.

MS: *m*/*z* = 227 (M^+), 180, 155, 152, 42.

HRMS: *m*/*z* calcd for $C_{10}H_{13}NO_5$ 227.0794, found 227.0792.

Anal. calcd for $C_{10}H_{13}NO_5$: C 52.86; H 5.77; N 6.17; found: C 52.97; H 5.84; N 6.20.

Ethyl 4-[(*tert*-Butyloxycarbonyl)amino]-3-methoxy-1-methyl-1*H*-pyrrole-2-carboxylate (7)

Diphenylphosphoryl azide (10.0 mL, 46.5 mmol) was added dropwise to an ice-cold slurry of **12** (10.0 g, 44.0 mmol) in anhyd MeCN (44.0 mL) and Et_3N (6.5 mL, 46.5 mmol). The resulting mixture was heated at 85°C for 2 h under argon, then it was cooled to r.t., treated with *t*-BuOH (88.0 mL), and again heated at 100°C for another 17 h, with good stirring. The cooled mixture was concentrated and the residue was partitioned between Et_2O (100 mL) and aq 10% Na_2CO_3 solution (50 mL). The layers were separated and the aqueous layer was extracted with Et_2O (2 × 20 mL). The combined extracts were dried (Na_2SO_4) and concentrated. Chromatographic purification (150 g of silica gel, 15% EtOAc in hexane) of the residue furnished 9.2 g (70%) of pure **7** as an oil.

^{1}H NMR: δ = 1.34 (t, *J* = 7.35 Hz, 3 H), 1.47 (s, 9 H), 3.75 (s, 3 H), 3.79 (s, 3 H), 4.29 (q, *J* = 7.35 Hz, 2 H), 6.29 (br s, 1 H), 7.00 (br s, 1 H).

^{13}C NMR: δ = 14.39, 28.35, 37.59, 59.77, 62.72, 80.31, 110.74, 114.87, 117.46, 129.85, 142.40, 153.00, 160.57.

IR (neat): ν = 3338, 2979, 1696, 1598 cm^{-1}.

MS: *m*/*z* = 298 (M^+), 283, 242, 198, 180.

HRMS: *m*/*z* calcd for $C_{14}H_{22}N_2O_5$ 298.1529, found 298.1530.

Ethyl 4-[(Allyloxycarbonyl)amino]-3-methoxy-1-methyl-1*H*-pyrrole-2-carboxylate (6)

The reaction was carried out as described above by starting with acid **12** (2.0 mmol) and by using allylic alcohol (2.0 mL) to intercept the isocyanate. Chromatographic purification (25 g of silica gel, 15% EtOAc in hexane) of crude material yielded 452 mg (80%) of **6** as an oil.

^{1}H NMR: δ = 1.36 (t, *J* = 7.35 Hz, 3 H), 3.78 (s, 3 H), 3.80 (s, 3 H), 4.31 (q, *J* = 7.35 Hz, 2 H), 4.62 (d, *J* = 5.9 Hz, 2 H), 5.24 (dd, *J* = 1.5, 10.3 Hz, 1 H), 5.34 (dd, *J* = 1.5, 17.7 Hz, 1 H), 5.86−6.10 (m, 1 H), 6.48 (br s, 1 H), 7.02 (s, 1 H).

^{13}C NMR: δ = 14.36, 37.64, 59.79, 62.72, 65.97, 110.87, 114.38, 117.66, 118.14, 132.53, 142.58, 153.38, 160.49.

IR (neat): ν = 3321, 2983, 1695, 1599 cm^{-1}.

MS: *m*/*z* = 283, 282 (M^+), 197, 179, 151.

HRMS: *m*/*z* calcd for $C_{13}H_{18}N_2O_5$ 282.1215, found 282.1208.

Ethyl 4-[(Benzyloxycarbonyl)amino]-3-methoxy-1-methyl-1*H*-pyrrole-2-carboxylate (14)

The reaction was carried out as described above by starting with acid **12** (2 mmol) and by using benzyl alcohol (1.0 mL) to intercept the isocyanate. Chromatographic purification (25 g of silica gel, 15% EtOAc in hexane) of crude material yielded 518 mg (78%) of **14** as an oil.

^{1}H NMR: δ = 1.35 (t, *J* = 7.35 Hz, 3 H), 3.77 (s, 3 H), 3.79 (s, 3 H), 4.30 (q, *J* = 7.35 Hz, 2 H), 5.16 (s, 2 H), 6.63 (br s, 1 H), 7.04 (s, 1 H), 7.30−7.41 (m, 5 H).

^{13}C NMR: δ = 14.35, 37.63, 59.79, 62.71, 67.15, 110.86, 114.37, 117.64, 128.23, 128.28, 128.42, 128.56, 136.12, 142.57, 153.49, 160.48.

IR (neat): ν = 3325, 2980, 1694, 1598 cm^{-1}.

MS: *m*/*z* 333, 332 (M^+), 224, 197, 151, 91.

HRMS: *m*/*z* calcd for $C_{17}H_{20}N_2O_5$ 332.1372, found 332.1368.

Anal. calcd. for $C_{17}H_{20}N_2O_5$: C 61.44, H 6.07, N 8.43; found C 61.47, H 6.27; N 8.27.

Benzyl 4-[(*tert*-Butyloxycarbonyl)amino]-3-methoxy-1-methyl-1*H*-pyrrole-2-carboxylate (15)

Compound **7** (7.5 g, 25.0 mmol) was added to an ice-cold solution of sodium (288.0 mg, 12.5 mmol) in benzyl alcohol (38.0 mL) and the resulting mixture was processed under vacuum as detailed earlier for **10**, except that heating at 100°C (10 Torr) was continued for only 1 h. The residue obtained upon removal of benzyl alcohol was partitioned between Et_2O (100 mL) and aq sat. NH_4Cl (20 mL). The layers were separated and the aqueous phase was washed with Et_2O (2 × 20 mL). The combined extracts were washed with brine (20 mL), dried (Na_2SO_4) and evaporated. Chromatographic purification (100 g of silica gel, 15% EtOAc in hexane) of the residue afforded 7.9 g (87%) of **15** as an oil.

^{1}H NMR: δ = 1.49 (s, 9 H), 3.73 (s, 3 H), 3.78 (s, 3 H), 5.31 (s, 2 H), 6.25 (br s, 1 H), 7.05 (s, 1 H), 7.30−7.50 (m, 5 H).

^{13}C NMR: δ = 28.27, 37.58, 62.75, 65.49, 80.21, 110.41, 114.89, 117.94, 127.98, 128.04, 128.44, 136.27, 142.75, 152.94, 160.19.

IR (neat): ν = 3335, 2977, 1695, 1597 cm^{-1}.

MS: *m*/*z* = 360 (M^+), 304, 260, 91, 57.

HRMS: *m*/*z* calcd for $C_{19}H_{24}N_2O_5$ 360.1685, found 360.1680.

HOAt Ester of 4-[(*tert*-Butyloxycarbonyl)amino]-3-methoxy-1-methyl-1*H*-pyrrole-2-carboxylic Acid (1)

A solution of **15** (7.2 g, 20.0 mmol) in dioxane (72.0 mL) containing 10% Pd/C (360.0 mg) was stirred under hydrogen pressure (40 bar) for 4 h. The catalyst was filtered off (cotton plug) and washed with dioxane (20 mL). Concentration of the combined filtrates under reduced pressure **at below 40°C** provided acid **16** in quantitative yield. This sensitive material was immediately taken up in anhyd CH_2Cl_2 (40 mL). The chilled (0°C) solution was treated with 4-dimethylaminopyridine (244.0 mg, 2.0 mmol), 1-hydroxypyridotriazole (HOAt, 4.1 g, 30.0 mmol) and DCC (6.2 g, 30 mmol), added in that order, with good stirring at 0°C under argon. The mixture was allowed to warm up to r.t. over 20 h, then it was filtered through a sintered glass funnel to remove the precipitate, which was washed

with additional CH_2Cl_2 (50 mL). The combined filtrates were washed with 0.05 N HCl (3 × 35 mL) and H_2O (3 × 30 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in a minimum amount of CH_2Cl_2 (5 mL). Addition of hexane (50 mL) induced precipitation of practically pure **1** (7.0 g, 90%); mp 158°C.

^{1}H NMR: δ = 1.44 (s, 9 H), 3.76 (s, 3 H), 3.98 (s, 3 H), 6.65 (br s, 1 H), 7.30−7.45 (m, 2 H), 8.39−8.41 (m, 1 H), 8.68−8.72 (m, 1 H).

^{13}C NMR: δ = 28.25, 37.66, 63.25, 80.69, 105.29, 115.94, 120.71, 122.92, 129.39, 134.99, 141.05, 145.21, 151.63, 152.93, 156.12.

IR (KBr): ν = 3235, 3090, 2975, 1761, 1714, 1602, 1554 cm^{-1}.

MS: *m*/*z* = 388 (M^+), 332, 253, 197, 125, 84.

HRMS: *m*/*z* calcd for $C_{17}H_{20}N_6O_5$ 388.1495, found 388.1484.

Anal. calcd for $C_{17}H_{20}N_6O_5$ C 52.57; H 5.19; N 21.64; found C 52.96; H 5.23; N 21.23.

Acknowledgement

We thank the CNRS (postdoctoral fellowship to K. N.), the MENRT, and the Région Rhône-Alpes for support of our research program. We are grateful to Dr. Daniela Fattori, of IRBM, S.p.A., Pomezia, Italy, and to her colleagues, for valuable discussion.

References

(1) (a) Herman, D. M.; Baird, E. E.; Dervan, P. B. *Chem. Eur. J.* **1999**, *5*, 975.
(b) Clairac, R. P. L.; Seel, C. J.; Geierstanger, B. H.; Mrksich, M.; Baird, E. E.; Dervan, P. B.; Wemmer, D. E. *J. Am. Chem. Soc.* **1999**, *121*, 2956.
(c) Trauger, J. W.; Baird, E. E.; Dervan, P. B. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 1421.
(d) Turner, J. M.; Swalley, S. E.; Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1998**, *120*, 6219.
(2) The synthesis of distamycin and related compounds has been summarized in an excellent review: Bailly, C.; Chaires, J. B. *Bioconjugate Chem*. **1998**, *9*, 513.
(3) (a) Van Dyke, M. W.; Hertzberg, R. P.; Dervan, P. B. *Proc. Natl. Acad. Sci. U. S. A.* **1983**, *79*, 5470.
(b) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *Proc. Natl. Acad.Sci. U. S. A.* **1985**, *82*, 1376.
(c) Klevit, R. E.; Wemmer, D. E.; Reid, B. R. *Biochemistry* **1986**, *25*, 3296.
(4) Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1996**, *118*, 6141.
(5) Dervan, P. B. World Patent WO 97/30975. *Chem. Abstr.* **1997**, *127*, 263060.
(6) Momose, T.; Tanaka, T.; Yokota, T.; Nagamoto, N.; Yamada, K. *Chem. Pharm. Bull.* **1978**, *26*, 2224.
(7) Ninomiya, K.; Shioiri, T.; Yamada, S. *Tetrahedron* **1974**, *30*, 2151.
(8) This sensitive material was not isolated.
(9) Gendler, P. L.; Rapoport, H. *J. Med. Chem.* **1981**, *24*, 33.
(10) Note added in proof. Subsequent to the acceptance of this manuscript, Dervan and collaborators reported full details of an alternative procedure for the large-scale synthesis of compounds related to the ones discussed in this paper: Urbach, A. R.; Szewczyk, J. W.; White, S.; Turner, J. M.; Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1999**, *121*, 11621.

Article Identifier:
1437-210X,E;2000,0,05,0673,0676,ftx,en;P05899SS.pdf