Total Synthesis of Antitumor Agent AT-125, (αS,5S)-α-Amino-3-chloro-4,5-dihydro-5-isoxazoleacetic Acid

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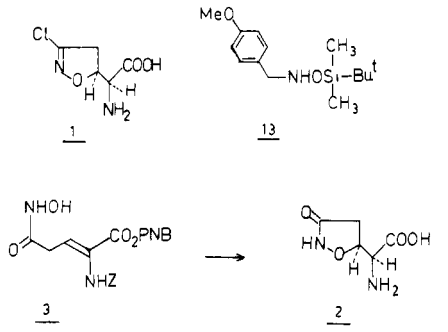
The antimetabolite AT-125 **1**,[1] from *Streptomyces sviceus*, has shown interesting antitumor activities in mice.[2] We wish to report a short and efficient, nonstereoselective, synthesis of AT-125.[3,4]

Originally we felt that tricholomic acid (**2**),[5] which in principle should be readily available from cyclization of a dehydroglutamic acid (**3**) (Scheme I), would provide a suitable intermediate for the synthesis of AT-125. In fact this approach was readily reduced to practice since the lactone acid **4**[6] after esterification with *p*-nitrobenzyl bromide (90%) was quantitatively isomerized with DBU in THF at 0 °C to **6**,[7,8b] mp 136–137 °C (Scheme II). Coupling of **6** with *O*-(*p*-nitrobenzyl)hydroxylamine, via the *N*-hydroxysuccinimide ester, was relatively clean (60%), and the product **7**[8c] (mp 158.5–159.5 °C) was easily deblocked with Zn(Cu) in DMF and benzenethiol[9] (45%). When this product, **8**[8d] (mp 130–132 °C, $CHCl_3$), was refluxed with 2.15 equiv of 1 N NaOH, the desired cyclization was achieved to yield a single isomer (86%) whose degradation, following Takemoto's method,[10] showed it to have the unnatural threo stereochemistry, as **9**.[8e] This disappointing stereochemical outcome is due to a net trans addition of the hydroxamate anion and a proton across the (*Z*)-olefin **8**. Preparation of the hydroxamic acid corresponding to **8**, having the *E* configuration, was not pursued since, even if available, it seemed likely to convert to **8** under the basic reaction conditions, prior to cyclization.

Scheme I[a]



[a] Z = carbobenzyloxy, NB = *o*-nitrobenzyl, and PNB = *p*-nitrobenzyl.

As a result of exploratory experiments, we established that the intramolecular Michael reaction on an ester substrate, as **11**, gave *both* diastereomers of the cyclic product **12**, presumably because of the formation of an intermediate enol. This reaction, however, only proceeded with N-substituted hydroxamic acid moiety **11** ($R^4 \neq H$). Consequently we decided to combine the required nitrogen substituent with one which would readily be a precursor for the imino chloride function at AT-125. Such a substituent was *p*-methoxybenzyl, and since N-substituted hydroxylamines are kinetically acylated on oxygen, the required reagent was, therefore, the *tert*-butyldimethylsilyl derivative **13**.[8i,11] The route was now straightforward (Scheme III). Coupling of dehydroglutamic acid **10**[8f] (mp 110–112 °C) with hydroxylamine **13** (via the *N*-hydroxysuccinimide ester), followed by removal (anhydrous KF in EtOH) of the substituted silyl group, gave the crystalline hydroxamic acid **11**[8g] (R^4 = PNB, R^5 = NB), mp 147–148 °C (50–60% from **10**), which was quantitatively cyclized with aqueous $NaHCO_3$ to a 1:1 mixture of *erythro*- and *threo*-**12** (R^4 = PNB, R^5 = NB). When subjected directly to a von Braun type dealkylation procedure (PCl_5 in refluxing CH_3NO_2), the substrate **12** gave the oximino chloride mixture (65%) which was separated by chromatography on silica gel (benzene–ether 9:1 v/v) into two substances, **14**[8j] (R_f 0.17) and its diastereomer (R_f 0.23). Deprotection of **14** with Al–Hg[12] gave cleanly **15**[8k] (70%) which was finally deprotected with $(COCl)_2$–DMF[13] in refluxing benzene (65%) to racemic AT-125 (**1**).[14] Optical resolution of racemic

(1) (a) L. J. Hanka and A. Dietz, *Antimicrob. Agents Chemother.*, **3**, 425 (1973); (b) D. G Martin, D. J. Duchamp, and C. G Chichester, *Tetrahedron Lett.*, 2549 (1973).

(2) (a) L. J. Hanka, D. G. Martin, and G. L. Neil, *Cancer Chemother. Rep.*, **57**, 141 (1973); (b) D. Hauchers, A. Ovejara, R. Johnson, A. Bogden, and G. Neil, *Proc. Am. Assoc. Cancer Res.*, **19**, 40 (1978).

(3) A somewhat lengthy, but stereospecific, synthesis has appeared: R. C. Kelly, I. Schletter, S. J. Stein, and W. Wierenga, *J. Am. Chem. Soc.*, **101**, 1054 (1979).

(4) Approaches to AT-125 by nitrile oxide cycloadditions have appeared. See: (a) J. E. Baldwin, C. Hoskins, and L. Kruse, *J. Chem. Soc., Chem. Commun.*, 795 (1976); (b) A. A. Hagedorn III, B. J. Miller, and J. O. Nagy, *Tetrahedron Lett.*, 229 (1980).

(5) T. Kamiya, *Chem. Pharm. Bull.*, **17**, 895 (1969), and references therein.

(6) A. E. Martell and R. M. Herbst, *J. Org. Chem.*, **6**, 878 (1941).

(7) The configuration of **6** was based on NMR chemical shift values with good precedent; cf. D. D. Keith, R. Yang, J. A. Tortora, and M. Weigele, *J. Org. Chem.*, **43**, 3713 (1978).

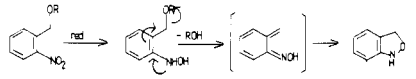
(8) (a) **5**: ^{1}H NMR ($CDCl_3$) δ 2.75 (s, 4 H), 5.08 (s, 2 H), 5.32 (s, 2 H), 6.37 (s, 1 H), 7.27 (s, 5 H), 7.45 (d, A_2 of A_2B_2, J = 9 Hz, 2 H), 8.14 (d, B_2 of A_2B_2, J = 9 Hz, 2 H); (b) **6**: ^{1}H NMR ($CDCl_3$) δ 3.33 (d, J = 7 Hz, 2 H), 5.11 (s, 2 H), 5.25 (s, 2 H), 6.80 t, J = 7 Hz, 1 H), 7.29 (s, 5 H), 7.44 (d, J = 9 Hz, 2 H), 7.98 (br s, 1 H), 8.12 (d, J = 9 Hz, 2 H); (c) **7**: ^{1}H NMR (acetone-d_6) δ 3.19 (br d, J = 7 Hz, 2 H), 5.04 (s, 2 H), 5.11 (s, 2 H), 5.35 (s, 2 H), 6.72 (br t, J = 7 Hz, 1 H), 7.31 (s, 5 H), 7.65 (d, J = 9 Hz, 2 H), 7.67 (d, J = 9 Hz, 2 H), 8.18 (d, J = 9 Hz, 4 H); (d) **8**: ^{1}H NMR (acetone-d_6) δ 3.14 (br d, J = 7 Hz, 2 H), 5.10 (s, 2 H), 6.71 (br t, J = 7 Hz, 1 H), 7.35 (s, 5 H), 7.7–7.9 (br s, 3 H); (e) **9**: ^{1}H NMR (acetone-d_6) δ 2.6 (br s, 1 H), 2.8 (br d, J = 8 Hz, 2 H), 4.58 (dd, J = 4 and 10 Hz, 1 H), 5.0–5.4 (m, 3 H), 6.8 (br d, J = 10 Hz, 1 H), 7.3 (s, 5 H), 8.25 (br s, 1 H); (f) **10**: ^{1}H NMR (acetone-d_6) δ 3.30 (d, J = 7 Hz, 2 H), 5.07 (s, 2 H), 5.51 (s, 2 H), 6.78 (t, J = 7 Hz, 1 H), 7.20 (s, 5 H), 7.46 (s, 3 H), 7.97 (m, 1 H), 8.70 (m, 1 H); (g) **11**: ^{1}H NMR ($CDCl_3$) δ 3.41 (d, J = 7 Hz, 1 H), 3.73 (s, 3 H), 4.68 (s, 2 H), 5.07 (s, 2 H), 5.58 (s, 2 H), 6.83 (d, J = 8 Hz, 2 H), 6.75–6.90 (m, 1 H), 7.19 (d, J = 8 Hz, 2 H), 7.28 (s, 5 H), 7.55 (m, 3 H), 8.09 (m, 1 H); (h) **12**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.75–2.96 (m, 2 H), 3.66 (s, 3/2 H), 3.71 (s, 3/2 H), 4.41–4.82 (m, 3 H), 4.95–5.12 (m, 1 H), 5.06 (s, 2 H), 5.25–5.34 (m, 2/2 H), 5.47–5.58 (m, 2/2 H), 6.74–6.79 (m, 2 H), 7.13–7.16 (, 2 H), 7.30 (s, 5/2 H), 7.33 (s, 5/2 H), 7.45–7.79 (m, 4 H), 8.10 (m, 1 H); (i) **13**: ^{1}H NMR ($CDCl_3$) δ 0.86 (s, 9 H), 3.70 (s, 3 H), 3.87 (s, 2 H), 4.95 (br s, 1 H), 6.75 (d, J = 8 Hz, 2 H), 7.13 (d, J = 8 Hz, 2 H); (j) **14**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.23–3.29 (m, 2 H), 4.62–4.69 (m, 1 H), 4.97–5.05 (m, 1 H), 5.13 (s, 2 H), 5.57 (s, 2 H), 5.84 (br d, J = 7 Hz, 1 H), 7.32 (s, 5 H), 7.45–7.69 (m, 3 H), 8.10 (m, 1 H); its diastereomer: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.08 (dd, J_{AB} = 18 Hz, J_{AX} = 8 Hz, 1 H), 3.22 (dd, J_{AB} = 18 Hz, J_{BX} = 11 Hz, 1 H), 4.62 (dd, J = 9.6 and 1.8 Hz, 1 H), 5.06 (s, 2 H), 5.24 (m, 1 H), 5.54 (s, 2 H), 7.25 (s, 5 H), 7.38–7.56 (m, 3 H), 8.02 (m, 1 H); (k) **15** ^{1}H NMR (300 MHz, acetone-d_6) δ 3.42 (dd, J_{AB} = 17 Hz, J_{AX} = 8 Hz, 1 H), 3.50 (dd, J_{AB} = 17 Hz, J_{BX} = 11 Hz, 1 H), 4.62 (dd, J = 9 and 4 Hz, 1 H), 5.10 (s, 2 H), 5.19 (ddd, 1 H), 6.92 (br d, 1 H), 7.35 (m, 5 H); (l) **16**: ^{1}H NMR (300 MHz, acetone-d_6) δ 3.47 (dd, J_{AB} = 17 Hz, J_{AX} = 7.7 Hz, 1 H), 3.55 (dd, J_{AB} = 17 Hz, J_{BX} = 11 Hz, 1 H), 4.20 (s, 2 H), 4.78 (dd, J = 8 and 4 Hz, 1 H), 5.15 (ddd, 1 H), 7.92 (br d, J = 8 Hz, 1 H).

(9) Procedure of Dr. L. D. Hatfield, Eli Lilly & Co., Indianapolis, Ind, to whom we are grateful for details.

(10) T. Takemoto and T. Nakajima, *Yakugaku Zasshi*, **84**, 1183 (1964).

(11) Prepared by $NaBH_3CN$ reduction (MeOH, pH 3) of *p*-methoxybenzaldoxime and subsequent silylation with *tert*-butyldimethylsilyl chloride (imidazole, DMF) in ~77% overall yield; bp 148–149 °C (1.4 mmHg).

(12) The mechanism for this deprotection is thought to involve initial reduction of the aromatic nitro group to the hydroxylamine, which subsequently undergoes benzylic cleavage. The resulting intermediate is trapped by the internal hydroxylamino nucleophile.



(13) (a) This deprotection involves sequential formation of an acid chloride followed by the Leuchs anhydride. This latter could be isolated but was most conveniently hydrolyzed directly to **1** with dilute HCl. (b) D. Konopinska and I. K. Siemion, *Angew. Chem., Int. Ed. Engl.*, **6**, 248 (1967).

(14) The 300-MHz (D_2O) NMR spectrum was identical with that of authentic AT-125: δ 3.30 (dd, A of ABX, J_{AB} = 18 Hz, J_{AX} = 8 Hz, 1 H), 3.39 (dd, B of ABX, J_{AB} = 18 Hz, J_{BX} = 11 Hz, 1 H), 3.91 (d, J = 3 Hz, 1 H), 5.15 (ddd, X of ABX, 1 H). Mass spectrum (chemical ionization) m/e 181, 179. Infrared spectra were identical. We thank Dr. R. C. Kelly of The Upjohn Co. for a sample of natural AT-125. 300-MHz (D_2O) NMR spectrum of *epi*-(*threo*)-AT-125: δ 3.45 (dd, A of ABX, J_{AB} = 18 Hz, J_{AX} = 7 Hz, 1 H), 3.59 (dd, B of ABX, J_{AB} = 18 Hz, J_{BX} = 11 Hz, 1 H), 3.90 (d, J = 7 Hz, 1 H), 5.13 (m, X of ABX, 1 H).

(15) S. Wolfe and M. G. Jokinen, *Can. J. Chem.*, **57**, 1388 (1979).

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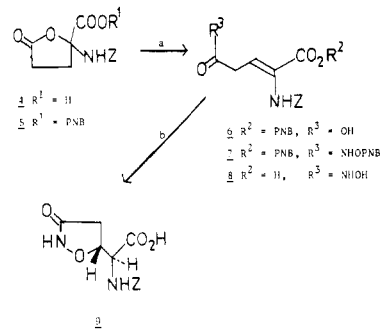
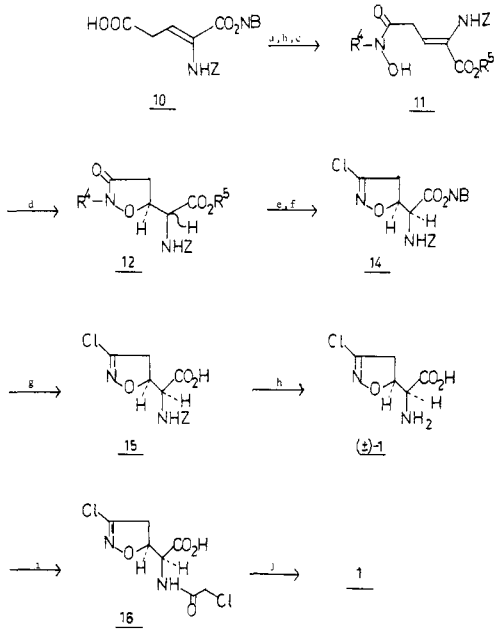
Scheme II[a]



[a] (a) DBU, THF, 0 °C; (b) 2.15 equiv of 1 N NaOH, reflux.

Scheme III[a]



[a] (a) DCC, *N*-hydroxysuccinimide, THF; (b) **13**; (c) anhydrous KF, EtOH; (d) dilute aqueous $NaHCO_3$; (e) PCl_5, CH_3NO_2; (f) column chromatography; (g) Al-Hg, ether; (h) $(COCl)_2$–DMF/benzene; (i) *p*-nitrophenyl chloroacetate;[15] (j) hog kidney acylase I.

1 was readily achieved via the chloroacetyl derivative **16**[8i] by means of hog kidney acylase I, affording optically pure AT-125 (57%), $[\alpha]^{20}_{378}$ +135° (c 0.159, H_2O).

This sequence involves eight separate steps, including deprotections, from the known and readily available **7**, and only one chromatographic separation, i.e., that of the oximino chloride **12** and its diastereomer.

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An Unusual Nucleophilic Attack on a Carbonyl Oxygen. Reaction of a Positively Charged Oxygen Atom

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Protonation on the nitrogen atom of *p*-benzoquinone monoimine (**1**) should give a species (**2**) with a positively charged oxygen atom,

Chart I

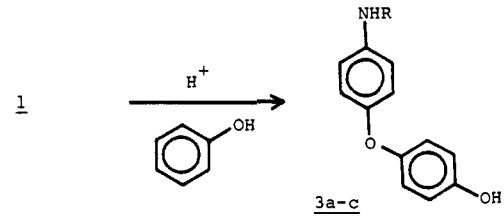
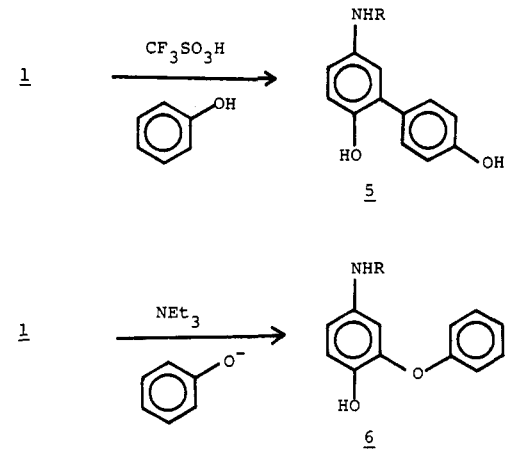
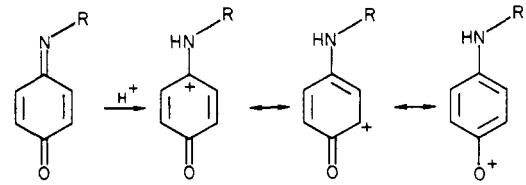


Chart II



a phenoxenium ion. In this paper we wish to report the discovery of a pathway which involves the species **2** in the reaction of *N*-acyl-*p*-benzoquinone imines (**1a–c**) with phenol, aniline, and dimethylaniline. This is an example of nucleophilic attack on an oxygen atom of a carbonyl group.



1a, R = SO_2CH_3
b, R = $COCH_3$
c, R = COPh-*p*-NO_2

2a-c

The chemistry of benzoquinone imines has been extensively studied by Adams.[1] The only known example of a reaction of the oxygen atom of **1** is the reaction with dialkyl phosphites.[2] Recently, attention has been paid to *N*-acetylbenzoquinone imine (**1b**), since the compound is believed to be a toxic reactive metabolite of phenacetin and phenacetamol.[3] It is clearly important to delineate the conditions under which nucleophiles react with the electrophilic compound.

The reaction of *N*-(methanesulfonyl)-*p*-benzoquinone imine (**1a**, R = Ms)[4,5] with excess phenol (20–100 equiv) in a solvent such as tetrahydrofuran, benzene, or methylene chloride proceeded smoothly at room temperature for 10 h (Chart I). The major product was 4-(methanesulfonylamino)phenyl 4-hydroxyphenyl ether (**3a**, R = Ms) isolated in 88% yield. The reaction site is

(1) Adams, R.; Reifschneider, W. *Bull. Soc. Chim. Fr.* **1958**, 23.
(2) Titov, E. A.; Avdeenko, A. P. *Obshch. Khim.* **1971**, *41*, 797; *Chem. Abstr.* **1971**, *75*, 63343, 76448.
(3) Calder, I. C.; Healey, K.; Yong, A. C.; Ham, K. N.; Yange, J. D. *Biol. Oxid. Nitrogen, Proc. Int. Symp., 2nd* **1978**, 308. Nelson, S. N. *Ibid.*, **1978**, 319. Calder, I. C.; Creek, M. J. *Aust. J. Chem.* **1976**, *29*, 1801. Shudo, K.; Ohta, T.; Orihara, T.; Nagao, M.; Takahashi, Y.; Sugimura, T. *Mutat. Res.* **1978**, *58*, 367.
(4) Adams, R.; Looker, C. R. *J. Am. Chem. Soc.* **1951**, *73*, 1145.
(5) All the new compounds were correctly analyzed and identified with authentic samples prepared by unambiguous reactions.

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