Total Synthesis of (8*R*)-3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol (Pentostatin), the Potent Inhibitor of Adenosine Deaminase[1a]

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A definitive synthesis for the potent adenosine deaminase inhibitor (8*R*)-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol (**1**)[1a] and its 8*S* isomer **13** is described, beginning with imidazole precursors. Construction of the heterocyclic portion **11** was accomplished by ring closure of the diamine **10** using triethyl orthoformate; **10** was prepared in eight steps from 4-methyl-5-nitroimidazole (**4**). Glycosylation of **11** was effected by using a low-temperature, stannic chloride catalyzed condensation of the pertrimethylsilyl derivative of **11** with a protected glycosyl halide.[1b] Subsequent separation of the anomeric nucleosides **12a** and **12b**, followed by deprotection and reduction of each, afforded the respective 8*R*,8*S* isomers **1**, **13**, and **14**. Of these, only the naturally occurring (8*R*)-β isomer **1** showed full inhibitory activity against adenosine deaminase.

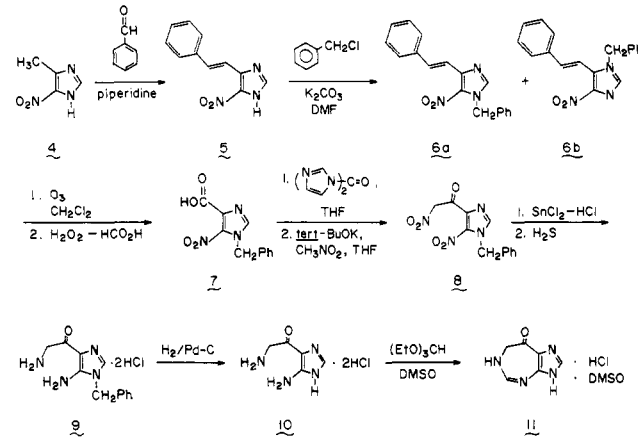
Over the past few years inhibitors of adenosine deaminase (adenosine aminohydrolase EC 3.5.4.4) have figured importantly as possible codrugs for use in combination with a number of adenine nucleosides in the treatment of both viral diseases and cancer.[2] Of these inhibitors, both pentostatin, (8*R*)-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol (**1**),[1,3] and coformycin (**2**), its D-*ribo* analogue,[4] are exceedingly tight-binding inhibitors of the enzyme, showing $K_i = 2.5 \times 10^{-12}$ and 1.0×10^{-11} M, respectively, against human erythrocytic adenosine deaminase.[5] Pentostatin (**1**), the tighter binding and more potent of the two, has shown dramatic antitumor effects when used in combination with 9-β-D-arabinofuranosyladenine (vidarabine) both in vitro[6] and in vivo.[7] The drug is currently under phase I clinical trials in combination with vidarabine against acute myelogenous leukemia.

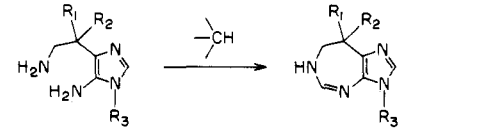
The structure of **1**, which was determined by single-crystal X-ray analysis,[3] presents a set of challenges to the synthetic chemist as there are in the molecule (1) a unique and unstable heterocyclic moiety, (2) a chiral alcoholic center in the aglycon, and (3) a 2-deoxy-β-D-*erythro*-pentofuranosyl moiety that defies the chemist's attempts to obtain a stereocontrolled glycosylation with a favorable β/α anomeric ratio. Our synthetic plans called for an acceptable solution to these problems that would provide a practical route to a multigram preparation of **1** for drug evaluation.

Results and Discussion

Synthesis of the Heterocyclic Moiety. The heterocyclic moiety, structurally a tetrahydroimidazo[4,5-*d*]-[1,3]diazepine, was envisioned as being synthetically derivable from a diamine precursor such as **3** that would possess either a ketone function (**3a**) or an alcohol group in the position α to the imidazole (**3b**). A removable protecting group (e.g., R_3 = benzyl) was deemed desirable for intermediate stages of the synthesis. It was anticipated that such a diamine could be ring closed with a suitable 1-carbon fragment, as with the few known examples of 1,3-diazepine syntheses,[8] to afford the basic heterocyclic system. Removal of the protecting group could be effected either before or after a ring closure was accomplished.

Scheme I





3 3a: R_1, R_2 = =O; R_3 = $-CH_2Ph$

3b: R_1, R_2 = —H, —OH; R_3 = $-CH_2Ph$

The early attempts to derive **3** from a number of known imidazole derivatives (e.g., 5-amino-1*H*-imidazole-4-carboxylic acid and 5-amino-1*H*-imidazole-4-carboxaldehyde, as well as the 5-nitro counterparts of these

(1) (a) For a preliminary communication, see: Baker, D. C.; Putt, S. R. *J. Am. Chem. Soc.* **1979**, *101*, 6127–6128. (b) Certain aspects of the glycosylation process have been communicated. See: Showalter, H. D. H.; Putt, S. R. *Tetrahedron Lett.* **1981**, *22*, 3155–3158.

(2) Shannon, W. M.; Schabel, F. M. *Pharmacol. Ther.* **1980**, *11*, 263–390.

(3) Woo, P. W. K.; Dion, H. W.; Lange, S. M.; Dahl, L. F.; Durham, L. J. *J. Heterocycl. Chem.* **1974**, *11*, 641–643.

(4) Ohno, M.; Yagisawa, N.; Shibahara, S.; Kondo, S.; Maeda, K.; Umezawa, H. *J. Am. Chem. Soc.* **1974**, *96*, 4327–4328.

(5) Agarwal, R. P.; Spector, T.; Parks, R. E., Jr. *Biochem. Pharmacol.* **1977**, *26*, 359–367.

(6) Cass, C. E.; Au-Yeung, T. H. *Cancer Res.* **1976**, *36*, 1486–1491.

(7) Cummings, F. J.; Crabtree, G. W.; Spremulli, E.; Rogler-Brown, T.; Parks, R. E., Jr.; Calabresi, P. *Proc. Am. Soc. Clin. Oncol.* **1980**, *21*, 332 (Abstract C-54).

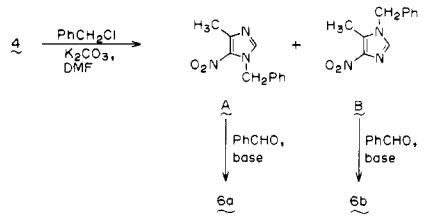
(8) deStevens, G. *Top. Heterocycl. Chem.* **1969**, 154–177.

0022-3263/82/1947-3457$01.25/0

compounds) by using standard processes of cyanohydrin synthesis, nitromethane condensation, and the like resulted at best in compounds of poor stability and limited synthetic potential. A finding that did emerge from these studies was that a compound of structure **3a** would be highly desirable, as the stability of such a compound, which is structurally a vinylogous amide, exceeds that of the corresponding vinylogous carbinol amines **3b**.[9] The idea of possibly introducing the chiral 8-OH group from an optically active precursor to give a **3b**-type compound of *R* stereochemistry was therefore abandoned.

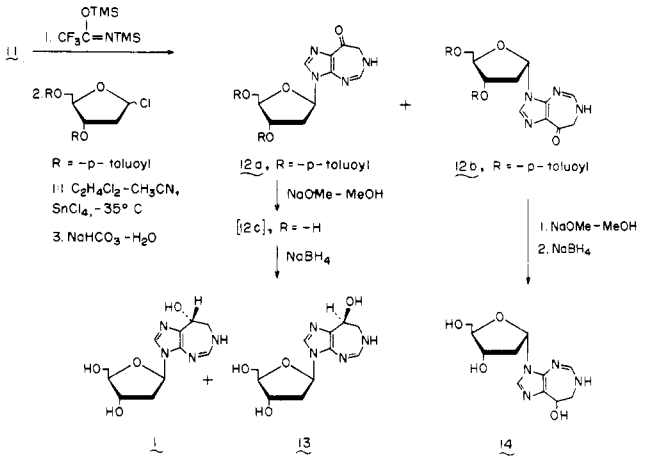
In order to develop a synthetically useful process to **3a**, we developed a route to the compound 2-nitro-1-(1-benzyl-5-nitro-1*H*-imidazol-4-yl)ethanone (**8**; see Scheme I) via the C-acylation of potassium methanenitronate with the imidazolide of 1-benzyl-5-nitro-1*H*-imidazo-4-carboxylic acid (**7**). This process,[10] demonstrated to be a generally useful route to a variety of α-nitro ketones, produced **8** as a stable, crystalline solid in 68% yield. Reduction of **8** with stannous chloride–concentrated hydrochloric acid then furnished the desired 2-amino-1-(5-amino-1-benzyl-1*H*-imidazol-4-yl)ethanone dihydrochloride (**9**; equivalent to **3a**) in 75% yield. Such reduction was found to be decidedly superior to catalytic techniques that invariably gave undesirable byproducts, the latter presumably arising from imcomplete reduction of the aliphatic nitro group. Debenzylation of **9** proceeded by a rapid and clean hydrogenolysis at pH $\leq$2 to afford 2-amino-1-(4-amino-1*H*-imidazol-4-yl)ethanone dihydrochloride (**10**) in near-quantiative yield.

The synthetic pathway to the diamine **10** is outlined in Scheme I. The starting material for the synthesis is 4-methyl-5-nitro-1*H*-imidazole (**4**)[11] that was converted by an improvement in the original process[12] to give 5-nitro-4-styrylimidazole (**5**). The latter compound was then benzylated in *N*,*N*-dimethylformamide–potassium carbonate to furnish in good yield a mixture of the isomeric *N*-benzyl derivatives **6a** and **6b** in a 3:1 ratio, respectively. Identification of each isomer was made by ^{1}H NMR, with the benzylic protons of **6a** exhibiting a shift (δ 5.58) to lower field than those for **6b** (δ 5.57), both separately and in admixture.[13] Ozonolysis of the mixture of **6a** and **6b** at −78 °C, followed by oxidation of the crude ozonides with cold performic acid, proceeded to give the pure 1-benzyl-5-nitro-1*H*-imidazol-4-carboxylic acid (**7**) that was



A (CH_2Ph, δ 5.51) and B (CH_2Ph, δ 5.29) are unambiguous; **6a** and **6b** so prepared were identical with samples produced by the alternate sequence (see Experimental Section).

Scheme II



easily separated from both its isomeric product and benzoic acid by a differential precipitation technique.

Ring closure to give 6,7-dihydroimidazo[4,5-*d*][1,3]-diazepin-8(3*H*)-one hydrochloride dimethyl sulfoxide (**11**) was carried out on the diamine **10** by using an excess of triethyl orthoformate in dry dimethyl sulfoxide at 65 °C to give directly an 81% yield of analytically pure **11**. The heterocycle invariably crystallized with inclusion of 1 mol of dimethyl sulfoxide, as revealed by both ^{1}H NMR and elemental analysis. The compound was found to be a highly insoluble species that rapidly reverted to **10** in water (as determined by TLC) and possessed a short, but finite, lifetime in absolute methanol, in which it could be dissolved for TLC analysis. That the 1-carbon fragment indeed had been incorporated into the diazepine ring was clearly shown the the appearance of a new, low-field ^{1}H NMR resonance at δ 8.21. The compound exhibited a distinctive new absorbance at 228 nm (ϵ 20900), with weak bands at 298 and 339 nm.

Condensation of the Heterocycle 11 with 2-Deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl Chloride. Of the numerous methods for glycosylation available to the chemist, nearly all methods were found to be too rigorous for the sensitive heterocycle **11**, either in its free form or as the pertrimethylsilylated derivative. Initially the best condensation conditions found were those involving pertrimethylsilylated **11** and 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl chloride in 1,2-dichloroethane in the absence of Lewis acids such as mercuric or stannic chloride, which, when present, invariably led to extensive degradation of the hererocycle.[1a] However, careful studies on the glycosylation process revealed that a low-temperature procedure,[1b] adapted from the stannic chloride catalyzed process of Vorbrüggen,[14] gave a 1:1 chromatographically pure mixture of 3-[2-deoxy-3,5-di-*O*-*p*-toluoyl-β(and α)-D-*erythro*-pentofuranosyl]-6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one [**12a** and **12b**, respectively (see Scheme II)] in a yield of 80–95%. A number of factors were found to be critical to the success of the condensation, among them the following: (1) a pure Me_3Si derivative of **11**, free of solvents and Me_3Si reagents, which is best formed a room temperature by using bis(trimethylsilyl)trifluoroacetamide; (2) an order of addition of reagents in which 1.5 molar equiv of anhydrous stannic chloride was added to the pertrimethylsilylated heterocycle at −35 °C, followed by addition of the halo sugar derivative at −45 °C and subsequent elevation of the temperature to −35 °C. Such a process produces products having neither

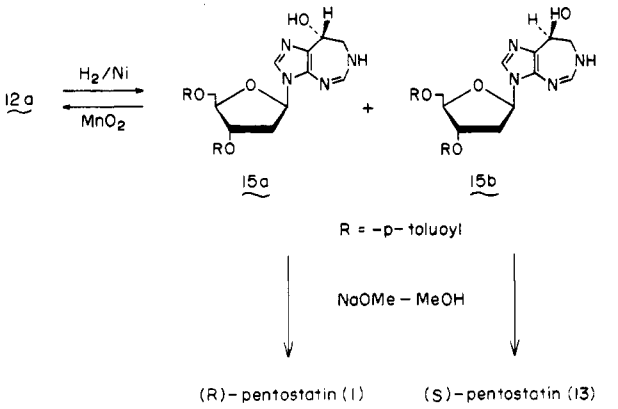
(9) Bernabe, M.; Burger, A. *J. Med. Chem.* **1971**, *14*, 883–885.
(10) Baker, D. C.; Putt, S. R. *Synthesis* **1978**, 478.
(11) 4-Methyl-5-nitroimidazole was supplied by BASF (Ludwigshafen, GFR). Alternatively, the compound could be synthesized via nitration of 4-methylimidazole (the latter prepared according to: Liggett, R. W.; Hoffman, H. L., Jr. U.S. Patent 3030376) by the procedure described by: Allsebrook, W. E.; Gulland, J. M.; Story, L. F. *J. Chem. Soc.* **1942**, 232.
(12) Windaus, A.; Langenbeck, W. *Ber.* **1923**, *56*, 683–686.
(13) Arguments can be made that the structural assignments for **6a** and **6b** are ambiguous, based on reasoning that the styryl group conjugation might complicate otherwise straightforward electronic effects of the nitro group on the CH_2Ph chemical shift. Therefore, the following chemistry was carried out (using conditions as for the analogous reactions in the Experimental Section). The ^{1}H NMR spectral assignments for
(14) Niedballa, U.; Vorbrüggen, H. *J. Org. Chem.* **1974**, *39*, 3654–3660.

detectable N-substituted isomers nor bis(glycosyl) adducts (as determined by TLC and ^{1}H NMR spectroscopy) as was observed with the original glycosylation procedure.[1a] Both **12a** and **12b** were found to be stable in a solution with protic solvents, relative to the parent heterocycle **11**.

In an attempt to overcome the problem of obtaining the anomeric mixture of nucleosides **12a** and **12b**, we tried a number of selectively derivatized glycosyl halides (i.e., those with a participating 3-*O*-acyl function and a nonparticipating 5-*O*-alkyl function),[15] without success, to obtain a good yield of the β anomer. Therefore, at this point the process whereby the anomeric nucleosides from the foregoing condensation of 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl chloride and the pertrimethylsilylated **11** were separated by ordinary column chromatography, or more expeditiously on large scale by a careful fractional crystallization to give the pure anomers **12a** and **12b**, was adopted. The β anomer **12a** exhibited a "pseudo" triplet at δ 6.46 ($J_{1',2'} \simeq J_{1',2'a} \simeq 7.24$ Hz) for H-1′ in the 200-MHz ^{1}H NMR spectrum (see Table I). Such a pattern (where $J_{1',2'} \simeq J_{1',2'a}$) has been shown to be characteristic[16] of a 2-deoxy-β-D-*erythro*-pentofuranosyl nucleoside, clearly distinguishing the β anomer **12a** from the α anomer **12b**. The latter showed H-1′ as a doublet of doublets at δ 6.53 ($J_{1',2'} = 7.0$ Hz and $J_{1',2'a} = 1.26$ Hz). The anomers **12a** and **12b** were also readily distinguished from one another by TLC, HPLC, and optical rotation.

Reduction of 12a to the 8*R* and 8*S* Alcohols 1 and 13. Inasmuch as natural pentostatin (1) is the 8*R* diastereomer, considerable effort was expended toward effecting a stereochemically favorable reduction of **12a** to give the (8*R*)-ol **1**. Sterically demanding borohydrides such as potassium tri-*sec*-butylborohydride and 9-borabicyclo-[3.3.1]nonane, as well as the modified lithium hydrides, lithium tri-*tert*-butoxyaluminum hydride, the lithium aluminum hydride–(−)-menthol complex,[17] and the lithium aluminum hydride–(−)-*N*-methylephedrine–3,5-xylenol complex,[18] gave either only marginal improvement in the 8*R*/8*S* ratio or low yields, or both. The most effective, albeit nonstereoselective, reducing agent proved to be sodium borohydride, and this reagent was employed on a preparative scale. Deprotection of the keto nucleoside **12a** via transesterification in methanol–sodium methoxide, followed by the addition of sodium borohydride in aqueous methanol, provided a ca. 1:1 ratio of the 8*R* and 8*S* isomeric alcohols **1** and **13** in 85% yield. Separation of these diastereomers up to a ca. 2–3-g scale was found to be best carried out by using preparative HPLC on an octadecylsilyl-derivatized silica gel column, operating in the reverse-phase mode. For larger scale work, the diastereomers are better separated by fractional crystallization, yielding the 8*R* and 8*S* components in high purity directly from the reaction mixture. By either of the above processes, **1** was isolated as an analytically pure material that was identical with the natural product[3] by melting point, TLC, HPLC, ^{1}H NMR, UV, and optical rotation. (8*S*)-3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol (**13**) is distinguished from **1** by its lower melting point, strongly negative optical rotation, and shorter HPLC retention time (see Experimental Section). The ^{1}H NMR spectra for **1** and **13** are quite similar, exhibiting only minor differences at 200 MHz (see Table I for ^{1}H NMR data).

Scheme III



In a manner identical with the foregoing, the α-anomeric alcohols **14** were obtained from the sodium borohydride reduction of **12b**. As these were shown to be of little biological interest (see Biological Evaluation section), the diastereomers were not separated.

An alternate reductive procedure was also found workable for the keto nucleoside **12a**. The ester-protected keto nucleoside 3-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8-(3*H*)-one (**12a**) could be reduced in 90% yield to a ca. 55:45 mixture of ester-protected 8*R* and 8*S* alcohols **15a** and **15b** by using commercial Raney nickel (see Scheme III). While the 8*S* isomer **15b** could be easily isolated pure, the 8*R* species **15a** defied all attempts at crystallization. Nevertheless, mixtures containing a ca. 75% enrichment of the desired 8*R* isomer could be routinely transformed into **1**. The undesired 8*S* isomer could be recycled to the keto nucleoside **12a**, albeit in low (15%) yield, by oxidation with activated[19] manganese dioxide.[20] The sodium borohydride reduction described in the foregoing section is, however, to be preferred for synthetic purposes.

Biological Evaluation.[21] The (8*R*)-β isomer **1** showed 100% of the expected activity against calf mucosal adenosine deaminase (vidarabine as substrate) when compared with the natural product. Both the (8*S*)-β anomer **13** and the (8*R*,*S*)-α anomeric mixture **14** were found to be less than 0.1% as potent as **1** on samples shown to be 100% nucleoside(s) by HPLC analysis. Since the binding properties of **1** are so profound ($K_i = 2.5 \times 10^{-12}$ M against human erythrocytic adenosine deaminase), even the slightest contamination of the products **13** and **14** by **1** could account for the observed activities. This point is the subject of further investigation.

Experimental Section

General Methods. Melting points were taken on a Thomas-Hoover Unimelt capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were determined on a Digilab FTS-14 instrument. Ultraviolet (UV) spectra were taken on a Cary Model 118C recording spectrophotometer. ^{1}H nuclear

(15) Baker, D. C.; Putt, S. R., unpublished work. For some related work, see: Wierenga, W.; Skulnick, H. I. *Carbohydr. Res.* **1981**, *90*, 41–52. Compare: Šmejkal, J.; Farkaš, J.; Šorm, F. *Collect. Czech. Chem. Commun.* **1966**, *31*, 291–297.

(16) Lemieux, R. U. *Can. J. Chem.* **1961**, *39*, 116–120.

(17) Andrisano, R.; Angeloni, A. S.; Marzocchi, S. *Tetrahedron* **1973**, *29*, 913–916.

(18) Vigneron, J. P.; Jacquet, I. *Tetrahedron* **1976**, *32*, 939–944.

(19) Fatiadi, A. *Synthesis* **1976**, 65.

(20) Various oxidants, including pyridinium chlorochromate (Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, 2647–2650) and dimethyl sulfoxide–oxalyl chloride (Mancuso, A. J.; Huang, S.-L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480–2482) as well as a number of other grades of manganese dioxide, were systematically evaluated for this oxidation. In all cases either incomplete oxidation was observed or intractable mixtures of decomposition products were obtained.

(21) We employed a modification of the procedure by: Mitchell, H. K.; McElroy, W. D. *Arch. Biochem.* **1946**, *10*, 343–349.

Table I. ^{1}H NMR Spectral Data for Compounds 1, 12, 13, and 15[a]

compd	chemical shift										
	H-2	H-5	H-7,7a	H-8	H-1′	H-2′,2a′	H-3′	H-4′	H-5′,5a′	*p*-toluoyl	other
12a[b,c]	7.74 (s)	7.46 (d,[d] $J_{5,NH}$ = 4.4 Hz)	3.98 (d, $J_{7,7a}$ = 3.68 Hz)		6.46 (t, $J_{1',2'}$ = $J_{1',2a'}$ = 7.24 Hz)	2.60–291 (m)	5.70 (m)	4.50–4.83 (m)		7.18–7.4, 7.80–8.18 (aryl), 2.39, 2.44 (s, CH_3)	1.57 (br s, NH)
12b[b,c]	7.87 (s)	7.46 (d,[d] $J_{5,NH}$ = 4.4 Hz)	3.97 (m)		6.53 (dd, $J_{1',2'}$ = 7.0 Hz, $J_{1',2a'}$ = 1.26 Hz)	2.69–2.76 (m), 2.96–3.10 (m)	5.69 (dd, $J_{2',3'}$ = 7.04 $J_{2a',3'}$ = 1.40 Hz)	4.87 (m, width 7.62 Hz)	4.58 (m)	7.19–7.38 (m), 7.70–7.98 (m, aryl), 2.36, 2.42 (s CH_3)	1.79 (br s)
12c[e]	7.92 (s)	7.53 (s)	4.03 (s)		6.35 (t, $J_{1',2'}$ = $J_{1',2a'}$ = 6.8 Hz)	2.71 (qt), 2.51 (ddd, $J_{2',2a'}$ = 13.8 Hz)	4.12 (dd, $J_{3',4'}$ = 4.0 Hz, $J_{2a',3'}$ = 3.9 Hz)	4.59 (qt, width 13 Hz)	3.81 (dd), 3.73 (dd, $J_{4',5'}$ = 3.6 Hz, $J_{4',5'}$ = 4.4 Hz, $J_{5',5a'}$ = 12.4 Hz)		
1[e]	7.70 (s)	7.20 (s)	3.52 (dd), 3.36 (dd, $J_{7,8}$ = 4.2 Hz, $J_{7a,8} \leq$ 1 Hz, $J_{7,7a}$ = 13.6 Hz)	5.15 (m, width ~4 Hz)	6.27 (t, $J_{1',2'}$ = 6.5 Hz, $J_{1',2a'}$ = 7.6 Hz)	2.69 (sp), 2.42 (ddd, $J_{2',2a'}$ = 14 Hz)	4.56 (qt, $J_{2a',3'}$ = 3.2 Hz, $J_{2',3'}$ = 6.4 Hz)	4.11 (q, width 10.4 Hz)	3.72 (dd), 3.78 (dd, $J_{4',5'}$ = 3.6 Hz, $J_{4',5a'}$ = 4.4 Hz, $J_{5',5a'}$ = 12.4 Hz)		
13[e]	7.68 (s)	7.19 (s)	3.53 (dd), 3.39 (dd, $J_{7,8}$ = 4.1 Hz, $J_{7a,8} \leq$ 1 Hz, $J_{7,7a}$ = 13.6 Hz)	5.16 (m, width ~4 Hz)	6.26 (t, $J_{1',2'}$ = 6.3 Hz, $J_{1',2a'}$ = 7.8 Hz)	2.73 (qt), 2.43 (ddd, $J_{2',2a'}$ = 13.9 Hz)	4.58 (dd, $J_{2a',3'}$ = 2.6 Hz) $J_{2',3'}$ = 7.8 Hz)	4.11 (m, width 10.5 Hz)	3.71 (dd), 3.79 (dd, $J_{4',5'}$ = 3.37 Hz, $J_{4,5a'}$ = 4.0 Hz, $J_{5',5a'}$ = 13 Hz)		
15a[b]	7.58 (s)	7.25 (s)	*f*	5.09 (t, width 7 Hz)	6.40 (t, $J_{1',2'}$ = 6.6 Hz, $J_{1',2a'}$ = 7.6 Hz)	2.66–2.82 (m)	5.69 (m, width 10 Hz)	4.52–2.63 (m)		7.92 (dd, (J_o = 10.98 Hz, J_m = 8.25 Hz), 7.19 (d), 2.39, 2.43 (s, CH_3)	2.5 (br s, NH)
15b[b]	7.56 (s)	7.24 (s)	*f*	5.10 (m, width 7 Hz)	6.41 (t. $J_{1',2'}$ = 6.6 Hz, $J_{1',2a'}$ = 7.6 Hz)	2.66–2.82 (m)	5.67 (qt, width 10.5 Hz)	4.52–4.63 (m)		7.92 (dd, J_o = 10.98 Hz, J_m = 8.25 Hz), 7.17 (d), 2.42, 2.38 (s, CH_3)	2.5 (br s, NH)

[a] Compounds are recorded at 200 MHz as ca. 1% w/v solutions in the indicated deuterated solvent with tetramethylsilane as the internal reference (δ 0.0). Both spin-spin coupling and chemical shift data (δ) are first-order approximations made from expanded (10 Hz/cm) data. Multiplicites: d, doublet; dd, doublet of doublets; ddd = doublet of (doublets); m, multiplet; q, four lines; qt, five lines; s, singlet; sp, seven lines. [b] In $CDCl_3$. [c] For a 90-MHz spectrum in methyl-d_6 sulfoxide, see ref 1. [d] Signal collapses to a singlet upon exchange of an NH proton in D_2O. [e] In D_2O. [f] Resonances overlapping with H-4′ and H-5′,5a′.

magnetic resonance (^{1}H NMR) spectra were recorded at 90 MHz on a Varian EM-390 or a Brüker WH-90 instrument or at 200 MHz on a Nicolet NT-200 instrument. Chemical shifts are reported as δ units downfield from internal tetramethylsilane on samples of ca. 1% w/v. Optical rotations were taken on a Perkin-Elmer 141 device. Combustion analyses were performed on a Perkin-Elmer 240 elemental analyzer. pK_a values were determined on a Copenhagen Radiometer TTT60 titrator.

Chromatography was carried out with (a) E. Merck products utilizing silica gel 60 (catalog No. 5760 for TLC, catalog No. 7734 for open column, and catalog No. 9385 for flash chromatography),[22] (b) Du Pont catalog No. 850952-701 for silica gel analytical HPLC, and (c) Altech catalog No. 600RP for C-18 reverse-phase analytical HPLC. Where specified, preparative separations with silica gel or C-18 reverse-phase separations were carried out on a Waters Associates Prep-500 system. Chromatography solvents include the following: A, 9:1 $CHCl_3$–MeOH; B, CH_2Cl_2; C, 3:1 acetonitrile–0.2 M NH_4Cl; D, 9:1 EtOAc–MeOH; E, 96:4 $CHCl_3$–MeOH; F, 9:1 0.005 M $(NH_4)_2HPO_4$–MeOH; G, 92.5:7.5 H_2O–MeOH, pH 7.5.

All solvents and reagents were "reagent grade" unless otherwise noted. When necessary, reaction solvents were dried prior to use either by distillation (THF from $NaAlH_4$; pyridine from CaH_2) or by storage over the appropriate activated Linde molecular sieves (nitromethane, 4A; Me_2SO, 5A; DMF, 4A; acetonitrile, 4A; 1,2-dichloroethane, 4A). All evaporations were conducted at 40–45 °C (10–20 torr) unless otherwise noted.

Abbreviations are as follows: DMF, *N,N*-dimethylformamide; MeOH, methanol; EtOAc, ethyl acetate; EtOH, ethanol; Me_2SO, dimethyl sulfoxide; THF, tetrahydrofuran.

Preparation of 5-Nitro-4-styryl-1*H*-imidazole (5). To 1.6 kg (12.6 mol) of 4-methyl-5-nitro-1*H*-imidazole (**4**)[11] in a 12-L flask equipped with a mechanical stirrer, thermometer, reflux condenser, and nitrogen inlet were added 3.65 L (3.81 kg, 35.9 mol) of benzaldehyde and 580 mL (488 g, 5.74 mol) of piperidine to form a thick slurry. The mixture was heated to 95 °C on a steam bath for 21 h, during which time a red solution formed and the product crystallized as a yellow solid. DMF (800 mL) and 4 L of 2-propanol were added, and the resulting slurry was heated at 95 °C for 1 h and then filtered hot. The filter cake was washed with 2-propanol (4 × 40 mL) and sucked dry, yielding 1.91 kg (70%) of analytically pure **5** upon drying at 90 °C (28 torr) for 18 h: mp 260 °C dec (lit.[12] mp 220 °C dec); R_f 0.3 (solvent A); ^{1}H NMR (Me_2SO-d_6) δ 7.15–7.57 (m, 8 H), 7.76 (s, 1 H); IR (KBr) 1635 (m), 1580 (m), 1560 (m), 1490 (s), 1350 (s), 1330 (s) cm^{-1}; UV (MeOH) 268 nm (ϵ 21 580), 367 (17 590).

Anal. Calcd for $C_{11}H_9N_3O_2$: C, 61.35; H, 4.22; N, 19.52. Found: C, 61.06; H, 4.21; N, 19.61.

An additional 127 g (4.7%) of **5** could be gleaned from the mother liquors by cooling to −20 °C for 8 h and filtering off the crystalline product. Long-term storage of the residues, diluted with 4 L of 2-propanol, yielded an additional 326 g of **5** (12%); total yield 2363 g (87%).

1-Benzyl-5-nitro-4-styryl-1*H*-imidazole and 1-Benzyl-4-nitro-5-styryl-1*H*-imidazole (6a,b). To 868.2 g (4.03 mol) of 4-nitro-5-styryl-1*H*-imidazole (**5**) and 841.5 g (6.1 mol) of anhydrous potassium carbonate in 9 L of DMF contained in a 22-L flask equipped with drying tube, dropping funnel and mechanical stirrer was added in one portion 553 mL (608 g, 4.8 mol) of benzyl chloride. The mixture was heated to 75 °C for ca. 6 h, at which time TLC (solvent A) revealed complete reaction.

The mixture was then cooled in an ice bath, and the salts were filtered and washed with CH_2Cl_2. The filtrate was evaporated at 50–60 °C ca. (1–2 torr) to give a yellow-orange oil, which was subsequently coevaporated with copious quantities of xylene to aid in removing traces of DMF. The oil was partitioned between ca. 12 L of H_2O and 6 L of CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), and evaporated to a yellow oil that solidified when dried at 25 °C (1 torr). The solid was triturated with cyclohexane, and the product was filtered and dried to give 1.18 kg (95.5%) of a mixture of ca. 75% **6a** and ca. 25% **6b** as determined by ^{1}H NMR: mp 92–94 °C; R_f 0.65 for **6a** and 0.60 for **6b** (solvent B). This product mixture was used directly in the next step.

For purposes of identification, the isomers **6a** and **6b** were separated by column chromatography (solvent B) to give pure **6a** (mp 105 °C), followed by **6b** (mp 127.5 °C).

Compound **6a**: ^{1}H NMR (Me_2SO-d_6) δ 5.58 (s, 2 H), 7.06–7.52 (m, 8 H), 7.52–7.77 (m, 4 H), 8.28 (s, 1 H); IR (KBr) 3130 (w), 1630 (m), 1510 (s), 1500 (s), 1475 (s), 980 (m) cm^{-1}; UV (MeOH) 272 nm (ϵ 24 274), 379 (15 328).

Compound **6b**: ^{1}H NMR (Me_2SO-d_6) δ 5.57 (s, 2 H), 7.09–7.60 (m, 12 H), 8.03 (s, 1 H); IR (KBr) 3110 (w), 1640 (w), 1540 (s), 1505 (s), 1490 (m), 1360 (s), 970 (m) cm^{-1}; UV (MeOH) 267 nm (ϵ 22 473), 355 (11 236).

Anal. Calcd for $C_{18}H_{15}N_3O_2$: C, 70.80; H, 4.95; N, 13.76. Found for **6a**: C, 70.79; H, 4.89; N, 13.67. Found for **6b**: C, 70.81; H, 4.94; N, 13.98.

1-Benzyl-5-nitro-1*H*-imidazole-4-carboxylic Acid (7). Into a 12-L, three-necked flask fitted with two ozone inlet tubes, a thermometer, a gas outlet tube, and a mechanical stirrer was dissolved 492.6 g (1.61 mol) of the crude mixture of isomeric benzyl compounds **6a** and **6b** in 6.6 L of 10:1 CH_2Cl_2–MeOH. The resulting solution was cooled in a dry ice–2-propanol bath to −78 °C, and ozone (generated in two Welsbach ozonizers, 0.06 CFM, 10 psi, dry oxygen stream) was bubbled in for ca. 8 h until a distinct blue color indicated excess ozone. The solution was slowly warmed to −30 °C under nitrogen purge to dispel the excess ozone, then concentrated to a pale yellow oil at 35 °C (**caution**: do not overheat nor evaporate to dryness!) and 1.52 L of 97% formic acid (prechilled to 0 °C) was added directly. To this solution, stirred and cooled in an ice bath, was added dropwise over 0.5 h 610 mL of a cold solution of 30% aqueous hydrogen peroxide. The cloudy solution was allowed to warm to room temperature over ca. 1 h, whereupon oxygen evolution began and the product began to separate. The exothermic reaction was moderated to the 20–60 °C range by cooling in an ice bath. After the reaction had subsided, the mixture was stirred overnight at room temperature. The precipitated solid was filtered, washed well with H_2O, and then suspended in 6.45 L of H_2O. The suspension was rapidly stirred and adjusted to pH 9.5 with concentrated NH_4OH, and the hazy mixture was filtered. The filtrate was adjusted to pH 5 with concentrated HCl, and 1.6 L of ether was added with vigorous stirring. The ether layer containing the benzoic acid byproduct was discarded, and the aqueous layer was acidified to pH 1.5 with concentrated HCl. The white precipitate was filtered off, washed with H_2O, air-dried at 25 °C for 24 h, and then thoroughly dried at 25 °C (5 torr) to yield 269.1 g (67%) of analytically pure **7**, free from both the isomeric acid and benzoic acid: mp 155–156 °C; pK_a (50% aqueous MeOH) = 3.5; ^{1}H NMR (Me_2SO-d_6) δ 5.42 (s, 2 H), 6.90–7.45 (m, 5 H), 8.16 (s, 1 H), 13.35 (br s, 1 H, CO_2H); IR (KBr) 3065 (m), 1725 (m), 1500 (s), 1360 (s), 840 (m) cm^{-1}; UV (MeOH) 291 nm (ϵ 4140).

Anal. Calcd for $C_{11}H_9N_3O_4$: C, 53.44; H, 3.67; N, 17.00. Found: C, 53.30; H, 3.80; N, 17.11.

All aqueous filtrates were combined, and the solution was adjusted to pH 1 and then extracted with EtOAc. Evaporation of the solvent gave a semisolid residue that was filtered and washed with copious amounts of H_2O to leave a solid that was 90% of the alternate isomer: pK_a (50% aqueous MeOH) = 2.2; ^{1}H NMR (Me_2SO-d_6) δ 5.40 (s, 2 H), 7.05–7.45 (m, 5 H), 8.08 (s, 1 H); IR (KBr) 3080 (m), 1720 (s), 1530 (s), 1395 (m) cm^{-1}.

2-Nitro-1-(1-benzyl-5-nitro-1*H*-imidazol-4-yl)ethanone (8). A mixture of 12.4 g (0.05 mol) of 1-benzyl-5-nitro-1*H*-imidazole-4-carboxylic acid (**7**) and 10.13 g (0.062 mol) of carbonyl-1,1′-diimidazole suspended in 110 mL of dry THF was heated under reflux until solution was complete (ca. 1 h).

To an ice-cold solution of 8.42 g (0.075 mol) of potassium *tert*-butoxide in 105 mL of dry THF was gradually added 14.9 mL (0.275 mol) of dry nitromethane. A white, gelatinous slurry resulted, and the solution of the imidazolide of **7**, prepared above and cooled to near 0 °C, was transferred rapidly under a nitrogen stream directly to the nitronate salt suspension which was stirred vigorously at 0–5 °C. The ice bath was subsequently removed, and the yellow solution was stirred for 0.75 h and poured onto ice. Water was added to dissolve the precipitate (solution pH 10.5). The aqueous solution was extracted with EtOAc (3 × 100 mL), and the aqueous layer was acidified to pH 2.5 with con-

(22) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.

centrated HCl. The solution was exhaustively extracted with EtOAc (all the while keeping the pH ≤3.0). The combined extracts were dried ($MgSO_4$) and evaporated to near dryness. Trituration in ether gave a light yellow solid that was filtered, washed well with ether, and dried at 25 °C (1 torr) to leave 9.92 g (68%) of analytically pure **8**, mp 108–110 °C. Crystallization from hot MeOH gave purified product: mp 113–114 °C; pK_a (50% aqueous MeOH) = 4.7; ^1H NMR (Me_2SO-d_6) δ 5.58 (s, 2 H, exchanges with D_2O), 6.21 (s, 2 H), 7.10–7.60 (m, 5 H), 8.36 (s, 1 H); IR (KBr) 1728 (s), 1560 (s), 1535 (s) cm^{-1}; UV (MeOH) 243 nm (ε 9650).

Anal. Calcd for $C_{12}H_{10}N_4O_5$: C, 49.66; H, 3.47; N, 19.31. Found: C, 49.59; H, 3.43; N, 19.27.

2-Amino-1-(1-benzyl-5-amino-1*H*-imidazol-4-yl)ethanone Dihydrochloride (9). To a solution of 514 g (2.28 mol, 6.6 equiv) of tin(II) chloride dihydrate in 600 mL of concentrated HCl contained in a 2-L flask was added portionwise, with ice-bath cooling and stirring, 100 g (0.345 mol) of 2-nitro-1-(1-benzyl-5-nitro-1*H*-imidazol-4-yl)ethanone (**8**), keeping the temperature below 50 °C. Approximately 40 mL of EtOH was used to transfer residual portions of the reagent. The solution was then heated to 60 °C for ca. 2.5 h, at which time TLC (solvent C) showed complete reaction of **8** (R_f 0.2) to form the tin(II) salt of **9** (R_f 0.0). The solvent was evaporated at 50 °C (20–30 torr) to give a yellow paste which was coevaporated with EtOH (3 × 150 mL). The resulting paste was poored into 6 L of ether with vigorous stirring, and the near-white precipitate of tin(II) salt was washed thoroughly with ether and then dried at 23 °C (5 torr).

The above solid tin(II) salt was dissolved in 1.75 L of H_2O in a 5-L, three-necked flask fitted with a mechanical stirrer, a gas inlet tube, and an outlet tube leading to a trap of 15% sodium hypochlorite solution (e.g., Chlorox). Hydrogen sulfide was bubbled in, and the tin(II) sulfide precipitate was filtered, yielding a clear filtrate. The solid cake was thoroughly washed with H_2O, and the combined filtrates were evaporated to dryness. The solid residue was coevaporated with EtOH as above and then was triturated with 200 mL of EtOAc. The white diamine dihydrochloride **9** was dried at 25 °C (1 torr). A second crop was recovered from the EtOAc triturate: yield 78 g (75%); mp 210 °C dec; R_f 0.55 (solvent C); ^1H NMR (Me_2SO-d_6) δ 4.14 (d, J = 6 Hz, 2 H; changes to s with D_2O), 5.31 (s, 2 H), 7.30 (s, 5 H), 8.34 (br s, overlapping s's, 5 H, NH's and H-2; changes to s with D_2O); IR (KBr) 1670 (s), 1630 (s), 1570 (m), cm^{-1}; UV (H_2O) 303 nm (ε 13 150).

Anal. Calcd for $C_{12}H_{13}N_4O$·2HCl: C, 47.54; H, 5.32; Cl, 23.39; N, 18.48. Found: C, 47.23; H, 5.40; Cl, 23.45; N, 18.57.

2-Amino-1-(5-amino-1*H*-imidazol-4-yl)ethanone Dihydrochloride (10). A 5-L, three-necked flask fitted with a fritted gas bubbler, a gas outlet tube (mercury bubbler), and a mechanical stirrer was charged with a solution of 50 g (0.165 mol) of 2-amino-1-(1-benzyl-5-amino-1*H*-imidazol-4-yl)ethanone dihydrochloride (**9**) in 2 L of 1:1 H_2O–MeOH. The solution was purged with nitrogen for 0.25 h, and 9 g of 10% palladium on charcoal, previously wetted with H_2O, was added. Hydrogen was vigorously bubbled into the solution with stirring, and the progress was monitored by TLC (solvent C). The acidity was maintained at pH ≤2 by addition of concentrated HCl as necessary, and the reaction was complete in ca. 16 h. The catalyst was filtered through Celite and washed with H_2O, and the combined filtrates were evaporated to dryness. The resultant light pink, granular solid was coevaporated with 150 mL of EtOH and then triturated with 30 mL of MeOH to give 34 g (96%) of pure, white product **10** after drying at 25 °C (1 torr): mp 260 °C dec; R_f 0.17 (solvent C); ^1H NMR (Me_2SO-d_6) δ 4.15 (d, J = 6 Hz, 2 H; changes to s with D_2O), 8.30 (br s, overlapping s, 6–7 H, NH's and H-2; changes to s with D_2O): IR (KBr) 1675 (s), 1625 (s), 1580 (s), 1450 (s) cm^{-1}; UV (MeOH) 233 nm (ε 2380), 301 (12 300).

Anal. Calcd for $C_5H_8N_4O$·2HCl: C, 28.18; H, 4.73; Cl, 33.28; N, 26.30. Found: C, 28.16; H, 4.74; Cl, 33.13; N, 26.19.

6,7-Dihydroimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one Hydrochloride Monodimethyl Sulfoxide (11). To a suspension of 205 g (0.96 mol) of 2-amino-1-(5-amino-1*H*-imidazol-4-yl)-ethanone dihydrochloride (**10**) in 5 L of dry Me_2SO was added, with stirring at 65 °C, 800 mL (713 g, 4.81 mol) of triethyl orthoformate. After ca. 0.25 h, a near-complete solution had resulted, and the insolubles were removed via filtration. The filtrate was decolorized with Darco G-60 and filtered through Celite, and the filtrate was evaporated to ca. one-third volume at 65 °C (1 torr). The concentrate was poured slowly into 12–24 L of vigorously stirred ether, whereby the product precipitated. The solid was filtered, washed well with ether, and dried at 60–80 °C (0.2 torr) for 16 h to give 105 g (81%) of analytically pure **10**: mp 153–155 °C; R_f 0.30 (solvent C); ^1H NMR (Me_2SO-d_6) δ 4.37 (s, 2 H), 8.05 (s, 1 H), 8.21 (s, 1 H), 12.1 (br s, NH's); IR (KBr) 1695 (s), 1645 (s), 1575 (s), 950 (m) cm^{-1}; UV (MeOH) 228 nm (ε 20 900), 298 (3460), 339 (2440); UV (MeOH + aqueous KOH) 241 nm (17 000), 328 (3620).

Anal. Calcd for $C_6H_6N_4O$·HCl·C_2H_6SO: C, 36.30; H, 4.95; N, 21.17, S, 12.11; Cl, 13.39. Found: C, 36.27; H, 5.02; N, 21.22; S, 21.10; Cl, 13.47.

3-[2-Deoxy-3,5-di-*O*-*p*-toluoyl-α(and β)-D-*erythro*-pentofuranosyl]-6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (12a,b). To a flame-dried, 5-L, three-necked flask fitted with a serum stopper, drying tube, and magnetic stirring bar were added 50.0 g (0.189 mol) of 6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8-(3H)-one hydrochloride monodimethyl sulfoxide (**11**), 153 mL (148.3 g, 0.576 mol) of *N*,*N*-bis(trimethylsilyl)trifluoroacetamide (Petrarch or Regis), 48 mL (0.594 mol) of dry pyridine, and 400 mL of dry acetonitrile (Burdick and Jackson spectroquality). The suspension was stirred at room temperature overnight, during which time a near-complete solution formed. Solvents and excess reagents were evaporated at 60 °C (1 torr), and 200 mL of acetonitrile was nitrogen transferred to the resulting syrupy product. The mixture was stirred to homogeneity, and the solvent was repeatedly added and evaporated in the above manner, keeping the residual syrup at ca. 60 °C until all volatiles had been removed, leaving a light brown solid residue.

The flask, fitted with a mechanical stirrer and dropping funnel, was charged with 1.2 L of acetonitrile, and the suspension was cooled to −35 to −40 °C. To the stirred suspension was added 33.85 mL (75.35 g, 0.289 mol) of anhydrous tin(IV) chloride (Baker or Ventron), whereby a solution formed after 8–10 min. The bath temperature was lowered to −45 to −50 °C, and a solution of 66.8 g (0.172 mol) of 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-pentofuranosyl chloride[23] in 1.2 L of dry 1,2-dichloroethane (Burdick and Jackson spectroquality) was added in one portion. The dark mixture was vigorously stirred for 0.75 h, during which time the temperature was slowly raised to −35 °C. [After ca. 10 min of reaction time, TLC (solvent D) revealed complete reaction.] The cold solution was poured into 3 L of a stirred solution of saturated aqueous $NaHCO_3$. About 1.5 L of EtOAc was added, the mixture was stirred for 0.5 h and filtered through Celite, the layers were separated, and the aqueous layer was extracted with EtOAc (2 × 700 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to ca. 150 mL. The white amorphous solid (primarily the α anomer **12b** by HPLC) was filtered off, and the filtrate was evaporated to a dark syrup that was flash chromatographed[22] over a 10 × 11 cm bed of silica gel with 94:6 EtOAc–MeOH (3 psi, 200-mL fractions). Appropriate fractions of the α,β-nucleosides **12b** and **12a** were combined, and the solvents were evaporated to give a residue that was triturated in hot CH_2Cl_2. The white solid was filtered off and was determined to be primarily the α anomer **12b** by HPLC. Trituration of the combined solids in 500 mL of hot $CHCl_3$ gave 16.35 g (17%; ~95% isomeric purity) of α anomer **12b**: mp 233–234 °C (with darkening at >215 °C); R_f 0.27 (solvent D); k' (HPLC, SiO_2, solvent E) = 3.4; $[\alpha]^{23}_D$ +7.1° (c 1, DMF); $[\alpha]^{23}_{436}$ +44.5°; for ^1H NMR data, see Table I; IR (KBr) 1720 (s), 1665 (s), 1610 (s), 750 (s) cm^{-1}; UV (MeOH) 235 nm (ε 50 235), 348 (3769).

Anal. Calcd for $C_{27}H_{26}N_4O_6$: C, 64.53; H, 5.22; N, 11.15. Found: C, 64.21; H, 5.09; N, 11.01.

The CH_2Cl_2 extract from the foregoing trituration was heated to boiling, and 500 mL of hot 1:1 MeOH–EtOAc was added, with vigorous boiling being maintained until crystallization set in. The mixture was slowly cooled to ca. 25 °C and then to 3 °C, and the resulting crystals were filtered with suction, giving 32 g of crude β anomer **12a**. Recrystallization was effected by dissolving the product in the absolute minimum of hot CH_2Cl_2, followed by the addition of 16 mL of 1:1 MeOH–EtOAc per gram of crude **12a** and cooling as above. By such a process was collected 23.05 g

(23) Bhat, C. C. *Synth. Proced. Nucleic Acid Chem.* **1968**, *1*, 521–522.

(23%) of pure β anomer **12a** as a methanolate: mp 151–153 °C; $[\alpha]^{23}_D$ −36° (*c* 1, DMF); R_f 0.27 (solvent D); *k'* (HPLC, SiO_2, solvent E) = 3.8; for ^{1}H NMR data, see Table I; IR (KBr) 1720 (s), 1660 (s), 1610 (s), 755 (s) cm^{-1}; UV (MeOH) 234 nm (ε 51 800), 350 (4050).

Anal. Calcd for $C_{27}H_{26}N_4O_6 \cdot CH_3OH$: C, 62.91; H, 5.66; N, 10.48. Found: C, 62.90; H, 5.73; N, 10.68.

By reprocessing the mother liquors of the above-purified nucleosides using the foregoing procedure, we obtained an additional 14.1 g (14.8%) of **12b** and 7.3 g (7%) of **12a**. Total yields: β-methanolate **12a**, 30.15 g (30%); **12b**, 30.45 g (32%).

(8*R*)- and (8*S*)-3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol (1 and 13). To a stirred suspension of 72 g (0.135 mol) of 3-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (**12a**)–methanol adduct in a 1.4 L of absolute MeOH was added 6.6 g (0.287 mol) of sodium over a period of 0.25 h. The compound gradually dissolved, and at the end of ca. 1 h, TLC (solvent D) indicated complete deprotection of **12a** (R_f 0.27) to give **12c** (R_f 0.03). Dry ice was added to neutralize the solution, and the solvent was evaporated to give a yellow residue which solidified upon trituration in ether. The solid was filtered, washed with ether, and used directly in the following step.

To the above solid dissolved in 425 mL of H_2O and 188 mL of MeOH was added 3.06 g (0.081 mol) of sodium borohydride, and the solution was stirred at ca. 25 °C for 0.5 h, at the end of which time the excess reducing agent was decomposed by the addition of dry ice. HPLC revealed a ca. 1:1 mixture of 8*R* and 8*S* isomers 1 and 13. The MeOH was evaporated, the hot aqueous solution was decolorized with 5 g of Darco G-60, and the solution was filtered and lyophilized to a fluffy solid. Trituration of the residue in 400 mL of hot MeOH, filtration, and washing of the inorganic solids with hot MeOH gave a clear filtrate that yielded a glassy syrup upon evaporation. The residue was dissolved in ca. 500 mL of H_2O, decolorized, and again lyophilized to a glassy solid. A second trituration in ca. 300 mL of hot MeOH gave solids that were filtered off, and the filtrate, upon concentration to ca. 160 mL and standing at 25 °C, gave crystals that were filtered and dried at 25 °C (1 torr) to leave 13.5 g of a 4:1 8*R*/8*S* mixture of isomers (1 and 13), as determined by HPLC.

The above diastereoisomeric mixture was dissolved in 15 mL of hot H_2O (pH 8.2, 85 °C), 10 mL of hot MeOH was added, and the suspension was filtered, washing the insolubles with 35 mL of hot MeOH. After being reheated to boiling, the solution was kept at 25 °C for 1 day and then at 3 °C for 3 days, giving white crystals that were filtered and washed with cold MeOH to give, after drying at 25 °C (1 torr), 9.36 g (26%) of a product that by HPLC was 97% 8*R* isomer 1. Purification over a C-18 reverse-phase column by preparative HPLC (solvent G) gave a product that by HPLC was >99% pure 8*R* isomer 1. Crystallization from H_2O–MeOH gave pure 1 that was homogeneous by HPLC: mp 204–209.5 °C, with darkening at >150 °C (lit.[3] mp 220–225 °C); $[\alpha]^{23}_D$ +73.9° (*c* 1, pH 7 buffer) (lit.[3] $[\alpha]^{23}_D$ +76.4° (*c* 1, H_2O); UV (pH 7 buffer), 282 nm (ε 8183) (lit.[3] 282 nm (ε 8000); *k'* (HPLC, C-18, solvent F) = 3.2; for ^{1}H NMR data, see Table I.

The 8*S* isomer was isolated via preparative HPLC as above from the mother liquors and precipitated by slow addition of a MeOH solution of the 8*S* isomer to excess either to give pure **13**: mp 130–140 °C dec; $[\alpha]^{23}_D$ −115° (*c* 1, pH 7 buffer); *k'* (HPLC, C-18, solvent F) = 2.1; pK_a (H_2O) = 5.6; for ^{1}H NMR data, see Table I; IR (KBr) 3600–3000 (br, s), 1630 (s), 1500 (m), 1100 (m), 1060 (m) cm^{-1}; UV (pH 7 or pH 11 buffer) 282 nm (ε 7920); UV (pH 2 buffer) 215 nm (ε 6110).

Anal. Calcd for $C_{11}H_{16}N_4O_4 \cdot 0.5CH_3OH$: C, 48.59; H, 6.38; N, 19.71. Found: C, 48.38; H, 6.57; N, 19.81.

Smaller scale preparations were most conveniently carried out as in the foregoing, with processing of the 8*R*/8*S* isomer mixture by preparative HPLC. In such a fashion, 10.25 g (0.02 mol) of **12a** gave 2.62 g (48%) of the 8*R* isomer 1, that was crystallized to give 1.83 g (33%) of analytically pure 1 (>99% by HPLC), and 2.19 g (40%) of the 8*S* isomer **13**, that was crystallized to give 1.67 g (29%) of pure **13** complexed with 0.5 mol of MeOH; mp 130–140 °C dec.

(8*R*)- and (8*S*)-3-(2-Deoxy-α-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ols (14). By the foregoing procedure for 1 and **13**, 400 mg (0.75 mmol) of 3-(2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl)-6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (**12b**) was deacylated by using sodium methoxide–methanol and reduced with 38 mg (1.0 mmol) of sodium borohydride in ethanol to give a mixture of the α-anomeric nucleosides **14** that by HPLC (C-18, solvent F) showed a ca. 60:40 mixture of nucleosides, having *k'* = 3.5 and 3.65, respectively. Purification and desalting of the crude product over a preparative C-18 column (1 × 40 cm) in the same solvent gave 165 mg (82%) of the nucleoside mixture **14**: $[\alpha]^{23}_D$ −22° (*c* 1, H_2O); UV (pH 7 buffer) 282 nm (ε 8100).

Anal. Calcd for $C_{11}H_{16}N_4O_4$: C, 49.25; H, 6.01; N, 20.88. Found: C, 49.42; H, 6.12; N, 20.76.

3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (12c). The triturate resulting from reaction of 9.68 g (0.018 mole of ketone **12a**, 250 mL of absolute MeOH, and 0.89 g of sodium as described above was paratitioned between H_2O and EtOAc. The aqueous layer was purified over a C-18 reverse-phase column by preparative HPLC (solvent G) to give a cream-colored solid. Trituration in ether left 4.3 g (85%) of product upon drying at 50 °C (1 torr) for 18 h: mp 117–119 °C; $[\alpha]^{23}_D$ −28.4° (*c* 1, pH 7 buffer); R_f 0.34 (solvent C); *k'* (HPLC, C-18, solvent F) = 2.9; IR (KBr) 1665 (s), 1600 (s), 1525 (s) cm^{-1}; UV (MeOH) 232 nm (ε 23 069), 299 (2975), 349 (3648); for ^{1}H NMR data, see Table I.

Anal. Calcd for $C_{11}H_{14}N_4O_4 \cdot 0.8H_2O$: C, 47.08; H, 5.60; N, 19.96. Found: C, 47.03; H, 5.27; N, 19.86.

(8*R*)- and (8*S*)-3-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol, (15a,b). A suspension of 5.025 g (10 mmol) of 3-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (**12a**), one heaping teaspoon of Raney nickel (K&K activated, catalog No. 27939; washed four times with distilled H_2O and then two times with MeOH), 450 mL of THF, and 50 mL of MeOH was stirred vigorously under 1 atm of H_2, with gradual warming from 5 to 25 °C over 17 h. The catalyst was filtered over Celite and thoroughly washed with MeOH. Evaporation of the filtrate left 4.6 g (91%) of a residual glass showing an 8*R*/8*S* ratio of 55:45 by HPLC. The glass was dissolved in 150 mL of hot ethyl acetate, and the solution was kept at 0 °C for 3 days. The crystalline 8*S* isomer **15b** was filtered, washed with cold ethyl acetate, and dried at 25 °C (1 torr) overnight to leave 990 mg of white, powdery 8*S* isomer **15b**: mp 199–200 °C; $[\alpha]^{23}_D$ −85.2° (*c* 1, MeOH); R_f 0.5; IR (KBr) 1725 (s), 1635 (s), 1275 (s), 755 (s) cm^{-1}; UV (MeOH) 239 nm (35 400), 282 (9400); UV (MeOH + 5 N HCl) 242 nm (ε 36 200); for ^{1}H NMR data, see Table I.

Conversion of either the pure 8*S* isomer or mixtures enriched in the 8*R* isomer to the corresponding isomers of pentostatin via sodium methoxide transesterification as described above was routinely carried out.

3-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (12a) via Manganese Dioxide Oxidation of (8*R*)- and (8*S*)-3-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydro[4,5-*d*][1,3]diazepin-8-ol (15a,b). A suspension of 500 mg (0.99 mmol) of **15a** and **15b** (84:16 8*S*/8*R* mixture), 2.5 g of activated MnO_2,[19] and 20 mL of pyridine was stirred at room temperature for 19 h. The mixture was filtered over Celite, concentrated at 40 °C (1 torr), and then coevaporated twice with toluene. Purification of the residue by silica gel flash chromatography,[22] with 3:97 MeOH–EtOAc and then by 5:95 MeOH–EtOAc as eluants, gave 80 mg (15%) of the recrystallized product **12a** (mp 138–142 °C) identical in all respects with ketone **12a**.

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Registry No. 1, 53910-25-1; 4, 14003-66-8; 5, 6307-17-1; 6a, 72079-75-5; 6b, 72079-76-6; 7 (isomer 1), 69195-96-6; 7 (isomer 2), 82228-58-8; 8, 69195-97-7; 9, 69195-91-1; 10, 69195-92-2; 11, 71222-44-1; 12a, 72079-79-9; 12b, 72079-80-2; 12c, 69196-03-8; 13, 69196-04-9; (8*R*)-14, 82264-17-3; (8*S*)-14, 82264-18-4; 15a, 82228-59-9; 15b, 82228-60-2; benzaldehyde, 100-52-7; nitromethane, 75-52-5; triethyl orthoformate, 122-51-0; 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-D-*erythro*-pentofuranosyl chloride, 3601-39-6.

Michael Reactions in Aprotic Media. An Effective Method of Construction of α,α,β-Trisubstituted Ketones and Application to Natural Product Synthesis

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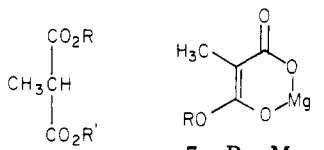
By proper selection of the reaction conditions, 1,4-additions of malonate and propionate anions to 2-(phenylthio)-2-cyclopentenone were accomplished. Subsequent trapping of the intermediate enolates with an electrophile in the aprotic media afforded α,α,β-trisubstituted ketones regiospecifically. The effects of the counterion, solvent, and temperature were examined. An application of this method to the formal synthesis of the pseudoguaianolides (±)-aromatin and (±)-confertin is described.

The Michael-type addition, defined as the nucleophilic addition of an anion to the carbon–carbon double bond of an α,β-unsaturated ketone, aldehyde, nitrile, or carboxylic acid derivative, has been extensively used as an effective method for carbon–carbon bond formation.[2] However, the conventional Michael reactions conducted in protic media exhibit several disadvantages[2a] such as the self-condensation of substrates, side reactions resulting from alkoxide anions, and the reversibility of reactions at high temperatures. These disadvantages can be circumvented by carrying out the addition reaction in an aprotic medium at low temperature[2b,c] with subsequent trapping of the intermediate enolate by an electrophile to form two new carbon–carbon bonds in a one-pot operation.

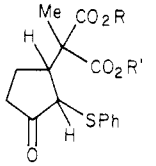
When an α,β-unsaturated ketone is employed in the reaction, the nucleophile can either add to the carbonyl center (1,2-addition) to provide alcohol products or add to the β-carbon (1,4-addition) to afford ketone products. It has been shown that the regioselectivity of addition is dependent upon reaction conditions such as temperature and solvent. For example, Schultz and Yee[3] have demonstrated that protonation of the reaction mixture of 2-cyclohexenone and the enolate of a 2-substituted propionate at −78 °C gives kinetic 1,2-adduct. However, stirring of the reaction mixture at 25 °C followed by protonation affords 1,4-adduct. Still[4] has shown that (trimethylstannyl)lithium adds to 2-cyclohexenone in the 1,2-fashion in diethyl ether but in the 1,4-fashion in tetrahydrofuran. Parallel results have been found in the addition of [(2-lithiophenyl)thio]acetonitrile to cyclic enones[5] and in the addition of [1,1-bis(methylseleno)-ethyl]lithium to 2-cyclohexenone.[6] The reactions[7] between (alkylthio)- or (phenylthio)allyl anions and 2-cyclopentenone have been shown to give predominantly 1,2-adducts in THF. However, generation of the anions in the presence of hexamethylphosphoric triamide (HMPA, 1 equiv) followed by the addition of 2-cyclopentenone furnishes exclusively 1,4-adducts. The preference of 1,4-ad-

Chart I

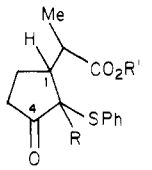


6a, R = R′ = Me
b, R = R′ = Et
c, R = Et; R′ = *t*-Bu

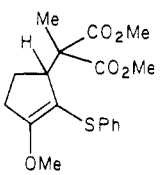
7a, R = Me
b, R = Et



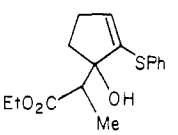
8a, R = R′ = Me
b, R = R′ = Et
c, R = Et; R′ = *t*-Bu
d, R = Me; R′ = H
e, R = Et; R′ = H



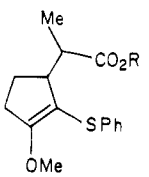
9, R = H; R′ = Me
10, R = H; R′ = Et
13, R = Me; R′ = Me
14, R = Me; R′ = Et



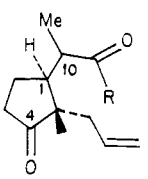
11



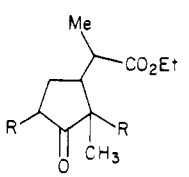
12



15a, R = Me
b, R = Et



16, R = OEt
17, R = OH
18, R = Cl
19, R = Me



20, R = H
21, R = CH_2CHCH_2

(1) Taken in part from the author's Ph.D. thesis, Yale University, 1981. Current address: Department of Chemistry, National Taiwan University, Taipei, Taiwan, Republic of China. The author is grateful to Professor Frederick E. Ziegler for his guidance throughout this work.

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