Contractile Activity of the Guinea Pig Gall Bladder. Solutions were prepared as described in the previous paragraph. The contractile activities were compared according to the procedure in ref 10.

Acknowledgment. This study was supported by grants from the U.S. Public Health Service (NIH AM-12573) and the Swedish Medical Research Council (B80-13X-01010-15A). Amino acid analyses was carried out by Delores J. Gaut, and elemental analysis by the Baron Consulting Co., Orange, Conn.

Potential Carcinostatics. 4.[1] Synthesis and Biological Properties of *erythro*- and *threo*-β-Fluoroaspartic Acid and *erythro*-β-Fluoroasparagine[2]

Martinus J. Wanner, Johanna J. M. Hageman, Gerrit-Jan Koomen, and Upendra K. Pandit*

Organic Chemistry Laboratory, University of Amsterdam, Nieuwe Achtergracht 129, Amsterdam, The Netherlands.
Received November 9, 1978

(*E*)- and (*Z*)-Di-*tert*-butyl 2-amino-3-fluoro-2-butene-1,4-dioate [(*E*)- and (*Z*)-**2**] were synthesized in two ways: (a) by elimination of hydrogen fluoride from di-*tert*-butyl β,β-difluoroaspartate under the influence of 1,5-diazabicyclo[4.3.0]non-5-ene and (b) by amination with the ammonium acetate of di-*tert*-butyl monofluorooxaloacetate (**3**), obtained via condensation of *tert*-butyl monofluoroacetate with di-*tert*-butyl oxalate. Reduction of **2** with sodium cyanoborohydride yielded a mixture of di-*tert*-butyl monofluoroaspartates in which the erythro isomer constituted the major product. The structure of this isomer (**4a**) was established by X-ray crystallographic analysis of the corresponding acid **5a**. Esterification of **5a** to the β-methyl ester **6**, followed by aminolysis, yielded *erythro*-β-fluoroasparagine (**7**). Tests with **5a** and **7** in the L-5178Y test system showed that the compounds exhibited toxicity at levels at which no antitumor activity was observed.

The major pathway for the biosynthesis of adenosine monophosphate (AMP)—a nucleotide essential for nucleic acid anabolism—involves the condensation of inosine monophosphate (IMP) with aspartate, to form adenylosuccinate, and subsequent loss of fumarate from the latter intermediate. We have recently directed our attention to the development of nucleoside analogues[3,4] which could act as inhibitors of adenylosuccinate synthetase (EC 6.3.4.4) and adenylosuccinate lyase (EC 4.3.2.2). An important target compound for this study is the adenylosuccinate analogue containing a stereospecifically located fluorine atom at the β position of the succinate moiety. Such an analogue could act as an inhibitor, especially of the lyase, if the elimination of fumarate from the adenylosuccinate proceeds via a stereospecific deprotonation step. For the synthesis of the aforementioned modified nucleotide, it is necessary to have convenient access to β-fluoroaspartic acids, which have been quoted to be unavailable on account of their instability.[5] This paper describes the synthesis of *threo*- and *erythro*-β-fluoroaspartic acid (**5a** and **5b**) and the conversion of **5a** to *erythro*-β-fluoroasparagine (**7**). The results of the preliminary biological investigation of **5a** and **7** are reported.

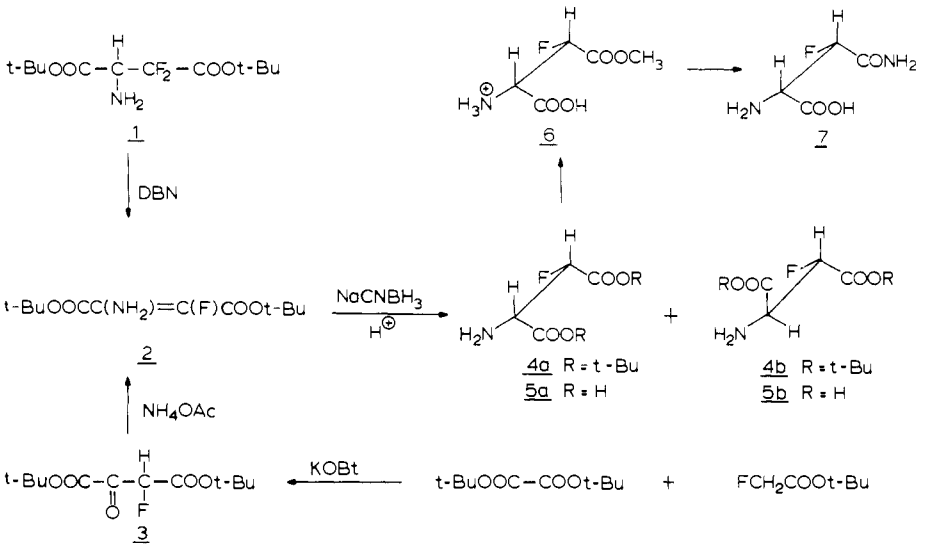
Chemistry. A practical approach to the synthesis of β-fluoroaspartic acid was conceived via the hydrogenation of β-fluoro-α-aminomaleic/fumaric acids (**2**). The latter precursor could be conveniently obtained by two routes (Scheme I). In one case, *tert*-butyl β,β-difluoroaspartate (**1**),[3] described by us recently, was subjected to dehydrohalogenation. While several bases could bring about the elimination of the elements of hydrogen fluoride, the best results were obtained by treating **1** with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN)[6] in THF, whereupon a mixture of the *Z* and *E* isomers of **2** (*Z*/*E* = 20:1; NMR) was obtained in 96% yield. In the second procedure, *tert*-butyl monofluoroacetate was condensed with di-*tert*-butyl oxalate, to yield monofluorooxaloacetate **3**,[7] which upon treatment with an excess of ammonium acetate (CH_3OH, room temperature, 4 days) gave an isomeric mixture of **2** (*E*/*Z* = 1:1, NMR) in a total yield of 85%. Attempts to catalytically hydrogenate **2** (isomeric mixture), however, led, in all cases, to the formation of di-*tert*-butyl aspartate. Presumably, the initially formed (desired) reduction product eliminates HF to give amino fumarate/maleate, which is reduced in a further hydrogenation reaction to the aspartate system.

The reduction of **2** with metal hydride reagents was investigated with a view to circumvent the problems associated with the catalytic reduction reaction. The use of cyanoborohydride in methanol/acetic acid, at room temperature, gave an isomeric mixture (**4a** + **4b**) of the expected di-*tert*-butyl β-fluoroaspartate contaminated with 5% of its cyanoborohydride complex and 5–10% of di-*tert*-butyl aspartate (the complex could be converted into a mixture of amino fumarate/maleate and *tert*-butyl β-fluoroaspartate upon heating in morpholine). The major isomer could be readily obtained in the pure form by crystallization. The minor product was isolated by laborious chromatography of the reaction mixture of a large-scale reduction of **2**. Since protonation of (*Z*)- and (*E*)-**2** leads to the same iminium salt (or its mirror image), the product distribution of **4a**/**4b** is independent of the composition of the starting mixture. NMR spectra of the individual isomers were insufficiently characteristic to allow their structural assignments (threo or erythro). The *tert*-butyl ester **4a** was conveniently converted into the

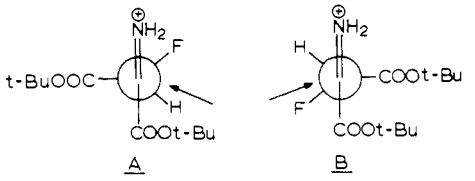
(1) For part 3, see W. M. Odijk, M. J. Wanner, G. J. Koomen, and U. K. Pandit, *Heterocycles*, **9**, 1403 (1978).
(2) Part of the forthcoming thesis of J. J. M. Hageman.
(3) J. J. M. Hageman, M. J. Wanner, G. J. Koomen, and U. K. Pandit, *J. Med. Chem.*, **20**, 1677 (1977).
(4) M. J. Wanner, J. J. M. Hageman, G. J. Koomen, and U. K. Pandit, *Recl. Trav. Chim. Pays-Bas*, **97**, 211 (1978).
(5) **Note added in proof**: Since the submission of this paper, the synthesis of DL-*threo*- and DL-*erythro*-β-fluoroaspartic acid without configurational assignment has been reported via a different synthetic route. [J. Kollonitsch, S. Marburg, and L. M. Perkins, *J. Org. Chem.*, **44**, 771 (1979)]. There has also been a comment on the presumed instability of β-fluoroaspartic acid [E. Kun, D. W. Fanshier, and D. R. Grasetti, *J. Biol. Chem.*, **235** 416 (1960)].
(6) H. Oediger, *Chem. Ber.*, 2012 (1966).
(7) D. E. A. Rivett, *J. Chem. Soc.*, 3710 (1953).

0022-2623/80/1823-0085$01.00/0

Scheme I



Scheme II



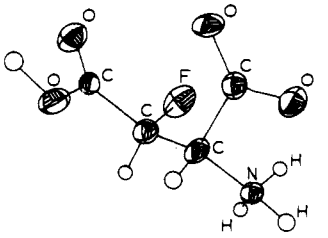


Figure 1. X-ray analysis of the free acid **5a** derived from **4a**.

corresponding acid **5a** by refluxing its solution in trifluoroacetic acid. The major reduction product was identified as the erythro ester **4a** on the basis of the X-ray analysis (Figure 1) of the free acid (**5a**) derived from it.

In order to explain the high stereoselectivity observed in the reduction of the iminium salt of **2**—formed under the acidic conditions of the reaction—the stereochemistry of the reduction was compared with that of the analogous α-halo ketones. The stereoselectivity of the reduction of α-halo ketones has been the subject of several studies.[8-11] Recently, Anh et al. have shown in a detailed molecular orbital study[12] that low energy transition states in such reactions are most effectively described by the Felkin model.[11] If this theory is applied to the intermediate iminium salts in the reduction of **2** (Scheme II), attack by the hydride should proceed antiperiplanar to the largest group. It has been convincingly argued that the attack is nonperpendicular, to the carbonyl, as a consequence of the interaction of the hydride's HOMO and the antibonding $\pi^*_{C=N^+}$ orbital. In the case of antiperiplanar attack in rotamer B, the transition state is destabilized by coulombic repulsion between the charged borohydride complex and the fluorine atom; this interaction suppresses the formation of the threo isomer.

erythro-β-Fluoroaspartic acid (**5a**) could be converted into the corresponding amide via a procedure which has been described earlier.[3] Treatment of **5a** with thionyl chloride/methanol produced the monomethyl ester **6** as its hydrochloride in 90% yield. A high ester carbonyl absorption at 1770 cm^{-1} indicated the presence of an α-fluorocarboxylic ester. Reaction with methanolic ammonia yielded *erythro*-β-fluoroasparagine (**7**).

Biological Results. *erythro*-β-Fluoroaspartic acid (**5a**) and the corresponding β-fluoroasparagine (**7**) have been examined for antileukemic activity in the asparagine-dependent L-5178Y lymphatic leukemia in vivo.[13] No antitumoral activity was observed for **5a** (NSC no. 300529) in doses up to 40 mg/kg. *erythro*-β-Fluoroasparagine (**7**; NSC no. 306904) was found to be inactive in doses up to 100 mg/kg. The amino acid analogue **5a** was also examined in the Lewis lung carcinoma test system. This system was chosen because of the recent finding of Stark et al.[14,15] that *N*-(phosphonoacetyl)-L-aspartate showed considerable activity in this system, whereas it proved to be ineffective in leukemia test systems. General toxicity prevented testing at doses higher than 12.5 mg/kg, at which concentration the compound was inactive.

Experimental Section

All melting points are uncorrected. IR spectra were recorded on a Unicam SP-200 spectrometer and NMR spectra were run on Varian Associates Model HA-100 and XL-100 instruments, using Me_4Si as an internal standard in the case of ^1H NMR spectra and $CFCl_3$ in the case of ^{19}F NMR spectra. Microanalyses were carried out by H. Pieters of the microanalytical department of this laboratory.

Antitumor Activity in Vivo. Lewis lung carcinoma homogenates (10^6 cells) were injected intramuscularly into six male BDF_1 mice.[14] Intraperitoneal treatment started 24 h after the inoculation of tumor cells. Animals received 11 injections from day 1 to 11. L-5178Y lymphatic leukemia (10^6 cells) were injected intraperitoneally into six female CDF_1 mice.[13] Intraperitoneal

(8) D. J. Cram and F. A. Elhafez, *J. Am. Chem. Soc.*, **74**, 5828 (1952).
(9) J. W. Cornforth, R. H. Cornforth, and K. K. Mathew, *J. Chem. Soc.*, 112 (1959).
(10) G. J. Karabatsos, *J. Am. Chem. Soc.*, **89**, 1367 (1967).
(11) M. Cherest, H. Felkin, and N. Prudent, *Tetrahedron Lett.*, 2201 (1968).
(12) N. T. Anh and O. Eisenstein, *Nouv. J. Chim.*, 1, 1 (1977).
(13) R. H. Adamson and S. Fabro, *Proc. Am. Assoc. Cancer Res.*, **9**, 2 (1968).
(14) R. K. Johnson, T. Inouye, A. Goldin, and G. R. Stark, *Cancer Res.*, **36**, 2720 (1976).
(15) K. D. Collins and G. R. Stark, *J. Biol. Chem.*, **246**, 6599 (1971).

treatment started 24 h after the inoculation of the tumor cells. Animals received nine injections from day 1 to 9. Cytostatic activity was determined by comparing median survival times with that of untreated control animals.

***tert*-Butyl β-Fluorooxaloacetate (3).** To a solution of 2.4 g of potassium in 57 mL of dry *tert*-butyl alcohol were added 12.8 g (64 mmol) of di-*tert*-butyl oxalate and 8 g (60 mmol) of *tert*-butyl fluoroacetate,[16] and the mixture was refluxed for 3 h. The yellowish-brown solution was cooled to room temperature, and 200 mL of 5% aqueous sodium hydroxide was added. The water layer was extracted with ether, and after acidification (pH 6, HCl) the product was obtained by ether extraction. Drying and evaporation of solvent produced 13 g (50 mmol, 83%) of **3** as a slightly colored oil: IR ($CHCl_3$) 3500 (OH, hydrated C=O), 1740 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.5 (*tert*-butyl), 5.02 (1, d, J_{H-F} = 47 Hz, C-H of the hydrated form), 5.72 (0.75 d, J_{H-F} = 48 Hz, C-H of free **3**).

(*E*)- and (*Z*)-Di-*tert*-butyl 2-Amino-3-fluoro-2-butene-1,4-dioate [(*E*)- and (*Z*)-2]. Method A. A solution of 15 g (53 mmol) of di-*tert*-butyl β,β-difluoroaspartate[3] (**1**) and 9.8 g (79 mmol) of diazabicyclo[4.3.0]nonene in 200 mL of tetrahydrofuran was refluxed for 5 h. After the solution was cooled, 300 mL of petroleum ether (bp 60–80 °C) was added and the decanted solution filtered through Hy-flow. Solvents were evaporated, and the residue was purified by column chromatography (silica; ethyl acetate—petroleum ether, 1:5). A mixture of *E* and *Z* isomers (*Z*/*E* = 20:1) was obtained as a colorless oil in 96% yield (13.5 g, 52 mmol).

Method B. A solution of di-*tert*-butyl fluorooxaloacetate (**3**; 4.8 g, 18 mmol) and 15 g (0.2 mol) of ammonium acetate in 75 mL of dry methanol was kept at room temperature until the starting material had been converted according to TLC (4 days). The solution was poured into 5% Na_2CO_3 solution and the mixture extracted with ether. Drying ($MgSO_4$) and evaporating the solvents produced 4.05 g of **2** (15 mmol, 85%) as a colorless oil (*Z*/*E* ≈ 1:1). (*E*)-**2**: IR ($CHCl_3$) 3500, 3380 (NH_2), 1710, 1680 (C=O), 1625 cm^{-1} (C=C); ^1H NMR ($CDCl_3$) δ 1.53 (18, s, *t*-Bu), 5.3–5.6 (2, NH_2). (*Z*)-**2**: IR ($CHCl_3$) 3520, 3420 (NH_2), 1740–1710 (C=O), 1655 cm^{-1} (C=C); ^1H NMR ($CDCl_3$) δ 1.55 (18, s, *t*-Bu), 4.0 (2, NH_2).

***erythro*- and *threo*-Di-*tert*-butyl β-Fluoroaspartate (4a and 4b).** The mixture of enamines **2** (12 g, 46 mmol) was stirred at room temperature with 3.2 g (50 mmol) of sodium cyanoborohydride in a mixture of 120 mL of dry methanol and 30 mL of dry acetic acid (distilled over P_2O_5) for 16 h. The solution was slowly added to 5% sodium carbonate and the water layer was extracted with ether. Combined ether extracts were dried ($MgSO_4$), the solvent was evaporated, and the residue was dissolved in petroleum ether (bp 60–80 °C). Cooling the solution yielded 5.0 g of **4a**. From the mother liquor an additional 1.6 g of **4a** and 0.02 g of **4b** were obtained by column chromatography (silica; petroleum ether–ethyl acetate, 2:1): total yield of **4a**, 6.69 g (25 mmol, 55%); mp 53–55 °C (petroleum ether); IR ($CHCl_3$) 3520 (NH_2), 1760–1730 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.43 (18, s, *t*-Bu), 1.80 (2, s, NH_2), 3.85 (1, d × d, J_{H-F} = 22, J_{H-H} = 2.5 Hz, C_α-H), 4.97 (1, d × d, J_{H-F} = 44, J_{H-H} = 2.5 Hz, C_β-H); ^{19}F NMR ($CDCl_3$) δ −91 (d × d, J_{H-F} = 47 Hz). Anal. ($C_{12}H_{22}FNO_4$) C, H, F, N. Total yield of **4b**, 0.02 g (1%); mp 73–75 °C (petroleum ether); IR ($CHCl_3$) identical with **4a**; ^1H NMR ($CDCl_3$) δ 1.48 (9, s, *t*-Bu), 1.51 (9, s, *t*-Bu), 1.65 (2, s, NH_2), 3.85 (1, d × d, J_{H-H} = 2, J_{H-F} = 31 Hz, C_α-H), 5.19 (1, d × d, J_{H-H} = 2, J_{H-F} = 29 Hz, C_β-H). Anal. ($C_{12}H_{22}FNO_4$) C, H, F, N.

***erythro*-β-Fluoroaspartic Acid (5a).** A solution of 3.7 g (14 mmol) of **4a** in 20 mL of trifluoroacetic acid was refluxed for 1 h. The solvent was evaporated and the residue was taken up in water. Upon addition of acetone, the free amino acid crystallized (1.6 g, 10.5 mmol, 75%): mp 174 °C dec; IR (KBr) 3600–2500 (COOH, NH_3^+), 1760 (NH_3^+), 1720 (C=O), 1600 cm^{-1} (COO^-); ^1H NMR (D_2O) δ 4.27 (1, d × d, J_{H-F} = 27, J_{H-H} = 2.5 Hz, C_α-H), 5.11 (1, d × d, J_{H-F} = 50, J_{H-H} = 2.5 Hz, C_β-H); ^{19}F NMR (D_2O) δ −193 (d × d, J_{H-F} = 28, J_{H-F} = 48 Hz). Anal. ($C_4H_6FNO_4$) C, H, F, N.

β-Methyl *erythro*-2-Fluoro-3-aminosuccinate Hydrochloride (6). To 1.5 mL of dry methanol, cooled to −18 °C, was added 0.27 mL of purified thionyl chloride. After the mixture reacted for 0.5 h, 0.5 g (3.3 mmol) of *erythro*-β-fluoroaspartic acid was introduced and the mixture was stirred at −18 °C for 2 h and at 20 °C for 1.5 h. Addition of 8 mL of dry ether produced 0.6 g (3.0 mmol) of the product as a white solid (90%): mp 167–170 °C (methanol/ether); IR (KBr) 3200–2500 (COOH, NH_3^+), 1770, 1750–1730 cm^{-1} (C=O); ^1H NMR (CD_3OD) δ 3.90 (3, s, CH_3), 4.77 (1, d × d, J_{H-H} = 2, J_{H-F} = 28 Hz, C_3-H), 5.58 (1, d × d, J_{H-H} = 2, J_{H-F} = 46 Hz, C_2-H). Anal. ($C_5H_9ClFNO_4$) C, H, F, N.

***erythro*-β-Fluoroasparagine (7).** In 100 mL of dry methanol, saturated with gaseous ammonia at 0 °C, 1.05 g (5 mmol) of monoester **6** was dissolved and the solution kept overnight at 4 °C, whereupon **7** crystallized. The mixture was stirred for an additional 5 h at 20 °C. Cooling to 4 °C and filtering produced 0.65 g (4.15 mmol) of **7** (83%) as a white solid: mp 210–213 °C dec; IR (KBr) 3400, 3200 (NH_2), 3000–2400 (COOH, NH_3^+), 1650, 1590 cm^{-1}; ^1H NMR (D_2O, NaOD) δ 3.83 (1, d × d, J_{H-H} = 2.5, J_{H-F} = 26 Hz, C_α-H), 5.15 (1, d × d, J_{H-H} = 2.5, J_{H-F} = 50 Hz, C_β-H). Anal. ($C_4H_7FN_2O_3$) C, H, F, N.

Acknowledgment. The authors express their gratitude to Drs. H. Schenk, K. Goubitz, and A. R. Overbeek of the Crystallographic Laboratory of the University of Amsterdam for the X-ray analysis of **5a** and to Dr. G. Atassi, Institut Jules-Bordet, University of Brussels, for the in vivo antitumor screening.

(16) E. D. Bergmann and S. Szinai, *J. Chem. Soc.*, 1524 (1956).

Synthesis and Antileukemic Activity of 1-Methyl-2,5-diphenyl-3,4-bis(hydroxymethyl)-, 1,2,3-Triphenyl-4,5-bis(hydroxymethyl)-, and 1-Methyl-2,3-diphenyl-4,5-bis(hydroxymethyl)pyrrole Bis(*N*-methylcarbamate)[1]

Wayne K. Anderson,* Michael J. Halat, and Arvela C. Rick

Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Amherst, New York 14260. *Received August 24, 1979*

The syntheses for the bis(*N*-methylcarbamates) **3**, **4a**, **4b**, and **5** are described. All four compounds were active in the in vivo P388 lymphocytic leukemia assay, with **3** being the most active.

In earlier reports, we described the preparation and antileukemic activity of 1-phenyl-2,5-dimethyl-3,4-bis(hydroxymethyl)pyrrole bis(*N*-methylcarbamate)[2] (**1**) and 1,2-dimethyl-3,4-bis(hydroxymethyl)-5-phenylpyrrole bis(*N*-methylcarbamate)[3] (**2**), as well as a series of ana-

(1) Vinylogous Carbinolamine Tumor Inhibitors. 6. For paper 5 in this series see: (b) Anderson, W. K.; McPherson, H. L., Jr.; New, J. S. *J. Heterocycl. Chem.*, in press.

(2) Anderson, W. K.; Corey, P. F. *J. Med. Chem.* **1977**, *20*, 1691.

0022-2623/80/1823-0087$01.00/0