Sephadex G-15 previously equilibrated with both phases of the solvent system n-BuOH-AcOH-H_2O (4:1:5). The column was eluted with upper phase at 10 ml/h and collected in fractions of 8 ml. The product was detected by monitoring the absorbancy at 280 nm. The fractions 68–73 were pooled with H_2O, the organic phase was removed in vacuum, and the aqueous phase was lyophilized. Yield 26 mg (12% overall yield based on initial phenylalanine attached to the polymer). The final product gave single spots on TLC (precoated plates of silica gel G) when loads of 10–15 µg were used, with R_f 0.16 (BAW), R_f 0.40 in the solvent system (upper phase) n-BuOH-AcOH-H_2O-pyridine (30:6:24:20) and R_f 0.53 in ethyl acetate-pyridine-AcOH-H_2O (5:5:1:3). Compound was detected on the chromatogram with ninhydrin, chlorine peptide spray and with diazotized sulfanilic acid. M.p. 217–219 °C (dec); $[\alpha]_D^{24}$ −55.3° (c 0.5, 1 N AcOH). Amino acid analysis gave the following molar ratios: Asp, 1.07; Arg, 0.96; Val, 1.03; Tyr, 0.90; Ile, 0.90; His, 0.90; Pro, 1.02; Phe, 1.05. Elemental analysis gave the following values: $C_{52}H_{76}N_{14}O_{11} \cdot C_2H_4O_2 \cdot 3H_2O$ (1187.36) calculated: C, 54.62; H, 7.31, N, 16.51; found: C, 54.40; H, 7.29; N, 16.45.

NMR-spectra run with a Varian HR-220 spectrometer at pHs 1.5, 7.5 and 9.0 indicated the analogue to be towards a trans-configuration around the His-Pro peptide bond.

Rat blood pressure test was performed according Regoli[11] and was about 70% relative potency to Hypertensin (Ciba) ([Asn^1]-angiotensin II). Rabbit aorta strips[12] gave intrinsic activity $a_E = 1$, a PD_2 of 6.92±0.09 and an affinity relative to [Asn^1]-angiotensin II of 6.5%. In both systems the action of the analogue was specific for the angiotensin II-receptor. Its action was competitively inhibited by [Leu^8]- and [Sar^1, Leu^8]-angiotensin II, while addition of a maximal dose of angiotensin II after maximal contraction caused by the analogue did not give any further contraction. As the relaxation time of the tissues after removing of the analogue was about as quick as for natural angiotensin II, it seems likely that it is degraded similarly to angiotensin II by aminopeptidases.

In conclusion it seems likely that the reduced affinity of the analogue tested should be explained by some steric hindrance of its N^4-dimethyl group in position 1, although modification of other type of interactions with the receptor (e.g. hydrogen bonding) cannot be completely excluded.

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A new synthesis of β-fluoroaspartic acid[1]

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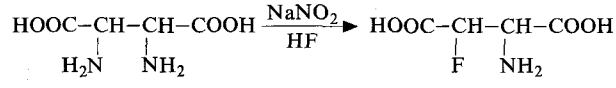
Summary. β-Fluoroaspartic acid, a new amino acid, was synthesized by a diazotization of diaminosuccinic acid in liquid hydrogen fluoride.

Halogenoamino acids, halogen-containing α-amino acids, have frequently been isolated from natural sources and have become attractive as a class of biologically interesting α-amino acids[3]. In particular, fluoro-α-amino acids are noteworthy because they act as metabolic antagonists to the naturally occurring α-amino acids[4], and show antibacterial activities against various microorganisms[5]. From this point, a considerable number of the fluoroamino acids have been synthesized; as monofluorinated α-amino acids, fluoroalanine, fluorobutyrine, fluorovaline, fluoroleucine, fluoroisolecine, fluorophenylalanine, fluorothreonine, fluoroproline, fluorohistidine, fluoroglutamic acid, etc., being reported[6–13].

Among these fluoroamino acids, however, fluoroaspartic acid[14], which is of great interest from the biological viewpoint, has never appeared as yet, though some attempts to synthesize have been made by several authors[8,9,15]. In this work, we have attempted the synthesis of fluoroaspartic acid[16] and found a convenient preparation of the desired β-fluoroaspartic acid from diaminosuccinic acid by the following scheme.



The process involves a simple diazotization in liquid hydrogen fluoride; the method and results are as follows. meso-Diaminosuccinic acid (14.8 g, 0.1 moles) was dissolved in liquid hydrogen fluoride (25 ml) kept at −30– −10 °C in a polyethylene bottle. To the mixture was added well dried sodium nitrite (6.2 g, 0.09 moles) in small portions for a period of 10 min under vigorous stirring. After the stirring

was continued for 1 h, hydrogen fluoride was removed in vacuo. To the residue was added 10% sodium carbonate solution (50 ml) and insoluble materials were filtered off. The filtrate was treated with an ion exchange resin, Amberlite IR 120 (H^+ form). The resulting amino acid was eluted with 1% hydrochloric acid and the eluate was evaporated to dryness under reduced pressure. The product was dissolved in water (10 ml) and the pH was adjusted to about 3 with ammonia. After standing overnight in a refrigerator, the resulting crystals were collected and washed with ethanol and then dried. The expected β-fluoroaspartic acid of 3.78 g (25%) was obtained. Structural identification of the amino acid was made by NMR-spectroscopy and elemental analysis as follows. M.p. 164–166 °C (dec.) (recrystallized from H_2O). ^1H NMR (60 MHz, D_2O+CF_3COOD), δ, 4.88 (d,d, 1H, α-CH, $J_{HH}=1.8$ Hz, $J_{HF}=28.8$ Hz), 5.62 (d,d, 1H, β-CH, $J_{HH}=1.8$ Hz, $J_{HF}=46.2$ Hz). Anal. Calculated for $C_4H_6O_4NF$: C, 31.79; H, 4.00; N, 9.27; F, 12.57; found: C, 31.99; H, 4.03; N, 9.52; F, 12.56. Paper electrophoresis (2000 V, buffer pH 3.8, 60 min): mobility = +7.9 cm. Paper chromatography (n-BuOH:CH_3COOH:H_2O = 5:3:1): R_f = 0.09. Although the stereochemistry of the resulting β-fluoroaspartic acid could not be determined from the available data, configuration of this amino acid would be *erythro*-form, judging from the nitrous acid deamination of α-amino acid which had generally been recognized to occur with retention of configuration[17]. In addition, the assumption was supported by finding a small amount of *erythro*-β-hydroxyaspartic acid as a by-product in the above reaction mixture on a paper electrophoresis. Further investigation of the stereochemistry and synthesis of the *threo*-isomer are currently in progress.

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Observation of pentacoordinated phosphorus intermediate in the reactions of nitrones with phosphonate anions

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Summary. The occurrence of a high-field signal in the ^{31}P FT NMR-spectrum of the reaction mixture of nitrones (*1* or *2*) and 2-cyanomethyl-4,5-dimethyl-2-oxo-1,3,2-dioxyphospholane (*7*), is interpreted in terms of a pentacoordinated phosphorus intermediate.

Previously we postulated that the reactions of nitrones (e.g. *1* or *2*) with carbanions of phosphonates[1,2,3] or phosphinoxides[4], that lead to aziridines (e.g. *3* or *4*) or to enamines (e.g. *5* or *6*), proceed via oxazaphospholidine intermediates (such as *8*) containing pentacoordinated phosphorus. In this communication we wish to present evidence that confirms this assumption.

Recently we reported that the reaction of the 5-membered cyclic phosphonate (*7*)[5] with *1* leads exclusively to aziridine *3a*[6], while that of *7* with *2* leads to the enaminonitrile *6a*[7]. In our quest for evidence concerning the existence of intermediate of type *8*, we focused our efforts on the reactions of this phosphonate (*7*), after failing to observe such an intermediate in reactions of open chain phosphonates and phosphinoxides. It is known that maximum stability of pentacoordination is attained when the phosphorus is part of a 'small' ring[8–13].

As experimental technique we have chosen ^{31}P FT NMR-spectroscopy (using a Bruker WP-60 instrument at 24.2 MHz). By this technique it is possible to observe species, even if they exist only as transient intermediates, provided that their steady-state concentration is sufficiently high. The steady-state concentration of *8* is proportional to $K_1/K_{-1}+K_2$ (see equation 1). Initial attempts to observe *8* were made by monitoring the reaction of *1* with *7* using sodium hydride in tetrahydrofuran at temperatures ranging from 0 °C to −60 °C, collecting spectra from 1000 scans at intervals of 10 °C (a fresh sample was used at each temperature). In these spectra we could observe signals at −30, −50 and −15 ppm (downfield from 85% H_3PO_4 as external standard) resulting from the phosphonate *7*[5], its anion and the cyclic phosphate *9*[14] respectively. The presence of the latter indicated that at all these temperatures the reaction proceeded. Following this we have considered that as the 1st step of the reaction is expected to be the rate-determining one, its activation energy will be higher than those of the 2nd step and of the reverse reaction (if there is such). Therefore the value of K_1 will be much more temperature-