Urea Cycle: Chemical Simulation of Arginine Biosynthesis†

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We have successfully simulated the salient features of the urea cycle. In this effort an appropriately protected ornithine was transformed to citrulline, via use of a novel amide transfer reagent; the citrulline, in turn, was converted into argininosuccinate which necessitated a new activation procedure to enable acceptance of dimethyl aspartate. Fragmentation of argininosuccinate under carefully controlled conditions afforded arginine. The final step in the cycle, namely, the hydrolysis of arginine to urea and ornithine, has already been accomplished. Amino group transfer from aspartate has also been demonstrated in the conversion of hypoxanthine to adenine.

The keen perception of Krebs and Henseleit[1] in 1932 that at least 30 urea molecules are formed at the expense of a single ornithine led to the identification of the latter as the carrier molecule in the urea cycle. The urea cycle, which plays a pivotal role in nitrogen metabolism, is initiated by amide transfer to the carrier molecule ornithine. The citrulline thus formed is activated to enable acceptance of aspartate leading to argininosuccinate, which on fragmentation via loss of fumarate results in arginine. The carrier molecule is regenerated by hydrolysis of arginine to urea and ornithine (Figure 1).[2]

Perhaps the most unusual aspect of the urea cycle is the manner by which nature transforms citrulline to arginine via the unique argininosuccinic acid. Apart from the fact that the choice of aspartate for nitrogen transfer is quite rare, this transformation requires the formation of a Schiff's base involving the highly unreactive urea carbonyl of citrulline.

There are only three known cases in nature where aspartic acid serves as the nitrogen donor. These are (i) the transformation of citrulline to arginine, (ii) the transformation of inosinic acid to adenylic acid, and (iii) the conversion of 5-amino-4-imidazolecarboxylic acid ribonucleotide to the corresponding 4-carboxamido nucleotide, an intermediate involved in the biosynthesis of purines.[3]

In each of the above cases the first step requires ATP activation of the substrate oxygen followed by condensation with the α-amino group of aspartate, giving rise to an intermediate which retains the carbon chain of aspartate. In the second step, detachment of the aspartate carbon chain occurs by C–N cleavage through β-elimination to fumarate and the aminated products.[4]

Results and Discussion

By far the most difficult task in the chemical realization of the salient features of arginine biosynthesis is the transformation of urea unit of citrulline to that of a substituted amidine. The first step here requires activation of the urea unit of citrulline, and this was attempted via transformation to either an isothiouronium salt, a carbodiimide, or a cyanamide. Of these only the cyanamide conversion proved successful.[5]

Thus, the reaction of N^{α}-(benzyloxycarbonyl)citrulline methyl ester (**1**) with *p*-toluenesulfonyl chloride in pyridine gave a quantitative yield of N^{α}-(benzyloxycarbonyl)-N^{δ}-cyanoornithine methyl ester (**2**). The generality of this reaction has been demonstrated by the transformation of N^{α}-benzoylcitrulline methyl ester (**5**) and N^{α}-benzoylcitrulline ethyl ester (**8**) to the corresponding cyanoornithines **6** and **9** in good yields (Figure 2).

Having accomplished the ureido group activation as the cyanamide unit, we explored the addition of elements of aspartic acid to give the desired argininosuccinate. In the event, it was found that the course of this addition is highly sensitive to the pH of the medium. When compound **2** was treated with the hydrochloride of dimethyl aspartate (**3**) in water at pH 1, the sole product isolated was N^{α}-(benzyloxycarbonyl)citrulline methyl ester (**1**), arising from hydrolysis of the cyanamide. On the other hand, treatment of **2** with freshly prepared **3** in water at pH 6 led to no reaction. The desired addition was eventually achieved via the reaction of **2** with the hydrochloride of **3** at pH 3.7 in MeCN–H_2O. Thus, the addition of an acetonitrile solution of **2** to a solution of the hydrochloride of **3** in water, adjusted to pH 3.7 with aqueous saturated solution of sodium bicarbonate, followed by reflux for 10 h and chromatography, provided the desired [N^{α}-(benzyloxycarbonyl)arginino]succinic acid trimethyl ester (**4**) in 40% yield. A careful chromatographic analysis of the reaction mixture reveals, in addition to **4**, N^{α}-(benzyloxycarbonyl)citrulline methyl ester (**1**) (24%), arising from hydrolysis, unreacted **2** (24%), and imidazolone **11** (1%) and imidazolinone imine **12** (5%), which arise via cyclization of the argininosuccinate **4**. The yield of **4** based on recovered **2** was 52%. The structural assignment for the key compound, the argininosuccinate **4**, is supported by spectral and analytical data.

The generality of the above procedure to prepare argininosuccinates from cyanamide precursors was further demonstrated via transformation of N^{α}-benzoyl-N^{δ}-cyanoornithine methyl ester (**6**) and N^{α}-benzoyl-N^{δ}-cyanoornithine ethyl ester (**9**) to **7** and **10** in respectively 60% and 55% yields (Figure 2).

The further transformation of argininosuccinate to arginine requires the loss of elements of fumarate. This process necessarily has to compete with alternate pathways, a major one arising from cyclization (vide supra). In view of the fact that amino group transfer from aspartic acid in the hypoxanthine → adenine conversion appeared relatively less complicated, parallel studies were undertaken to effect the chemical simulation of this reaction as well as that of the model system represented by the 4-oxoquinazoline → 4-aminoquinazoline transformation.

† Respectfully dedicated to Professor Sir D. H. R. Barton on the occasion of his 70th birthday.

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(4) Havir, E.; Hanson, K. *The Enzymes*, 3rd ed.; Boyer, P., Ed.; Academic Press: New York, 1973; Vol. 7, p 75.
(5) The reaction of monosubstituted ureas with *p*-toluenesulfonyl chloride has been reported to afford a complex mixture from which small amounts of *N*-tosylcyanamides could be isolated (Kurzer, F. *J. Chem. Soc.* **1949**, 3029). We have found that this reaction when carried out in pyridine leads to cyanamides in good to excellent yields.

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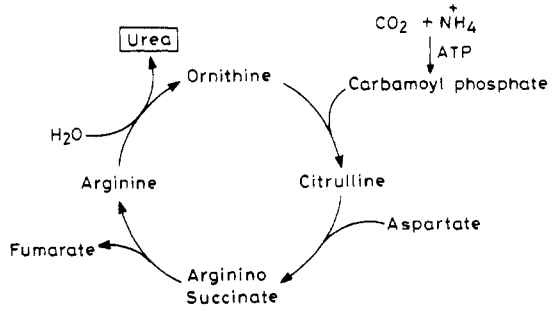

Figure 1. The urea cycle.

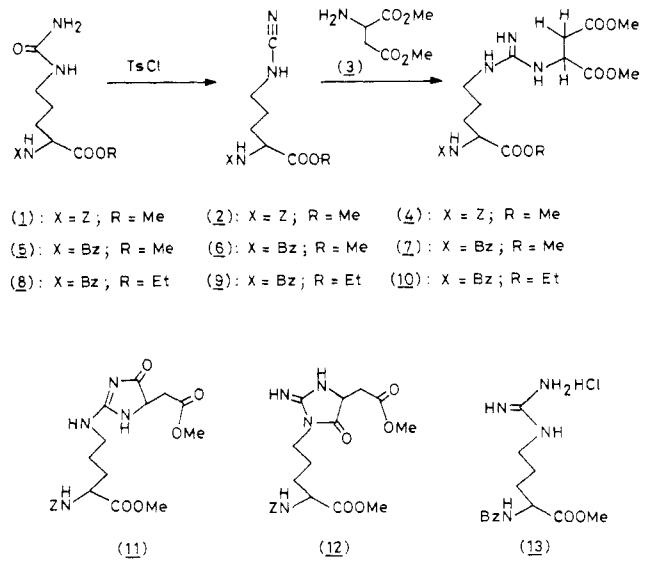

Figure 2. Preparation of cyanamides and argininosuccinates from citrullines.

The reaction of 4-oxoquinazoline (**14**) with phosphorus oxychloride afforded 4-chloroquinazoline, which on treatment with freshly prepared dimethyl aspartate (**3**) in refluxing xylene afforded the desired quinazolyl succinate **15** in fair yields. The structural assignment for **15** is fully supported by spectral and analytical data.

Hypoxanthine (**17**) was transformed to 6-chloropurine (PCl_5, $PhN(Me)_2$), 9-N-protected (K_2CO_3, DMSO, $PhCH_2Cl$), and the resulting 9-benzyl-6-chloropurine reacted with **3** in refluxing xylene for 8 h to afford the desired adenylosuccinate **18** (42%).

It appeared to us that the fragmentation of adducts such as **15** and **18** could be best effected via a reagent that could initially function as a proton donor with the resulting conjugate base capable of proton abstraction. Indeed, when a mixture of the adduct **15** and butyric acid in xylene was refluxed for 6 h, the anticipated fragmentation took place leading to the isolation of 4-aminoquinazoline (**16**) in 50% yield. The adenylosuccinate **18**, under similar conditions, gave a 42% yield of 9-benzyladenine (**19**), which was found identical with an authentic sample in all respects (Figure 3).[6]

Apart from its relevance to arginine biosynthesis, the transformation of 9-benzylhypoxanthine to 9-benzyladenine involving amino transfer from aspartic acid constitutes the first chemical simulation of this important biological reaction.

Although compounds **4**, **7**, **10**, **15**, and **18** are derived from aspartate and activated substrates, their fragmentation profiles differ. Thus, the butyric acid treatment

(6) Attempted thermal fragmentation of either **15** or **18** to respectively **16** and **19** did not succeed.

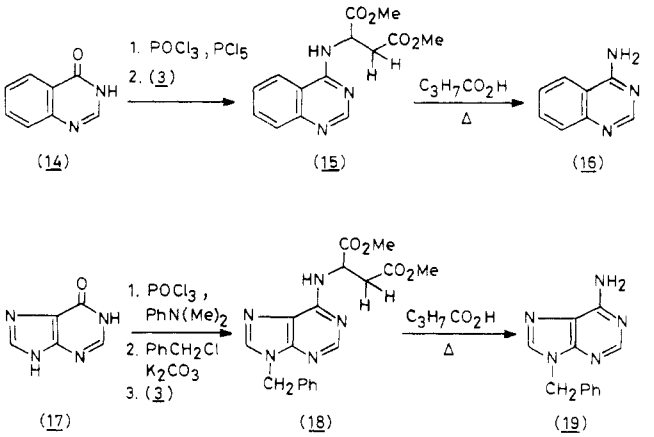

Figure 3. Transformation of 4-oxoquinazoline and hypoxanthine to, respectively, 4-aminoquinazoline and adenine.

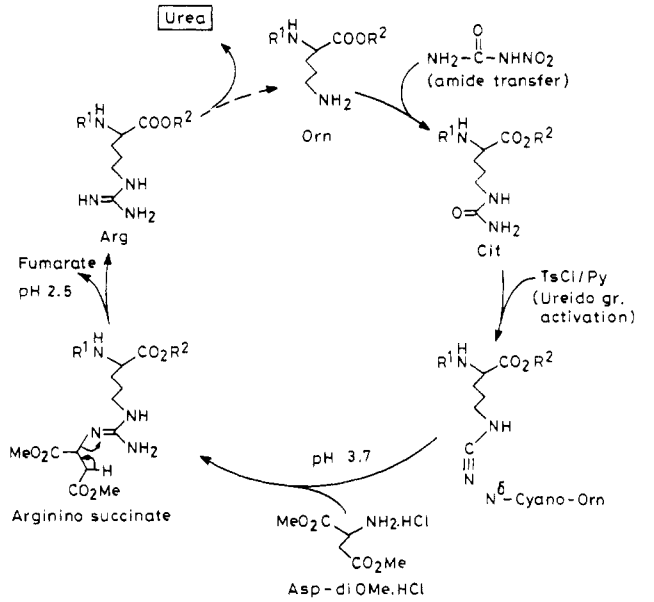

Figure 4. Chemical simulation of the salient features of the urea cycle.

which was so effective in the fragmentation of **15** and **18** was found to give complex mixtures when applied to the argininosuccinates **4**, **7**, and **10**. Similarly, whereas the quinazolyl succinate **15** could be transformed to 4-aminoquinazoline (**16**) (67%) in hot methanesulfonic acid, this treatment resulted in extensive decomposition of adenylosuccinate **18** or the argininosuccinates.

An extensive exploration was required to determine conditions under which argininosuccinate could fragment readily to arginine and fumarate. A fortuitous observation eventually led to the solution of this vexing problem. In one of the experiments, reaction of cyanamide **6** and hydrochloride of **3** at pH 2.5 gave, in addition to the expected argininosuccinate **7** (30%) and citrulline **5** (29%), the cleaved product, N^{α}-benzoylarginine methyl ester hydrochloride (**13**) in 19% yield. The argininosuccinate **7** when subjected to similar pH conditions in aqueous acetonitrile under reflux for 40 h gave the cleaved product **13** in 56% yield.

The ornithine → citrulline link was established via use of nitrourea as the carbamoyl transfer reagent. The copper complex of L-ornithine was reacted with an aqueous solution of nitrourea under reflux, the complex decomposed and N,C-protected to give N^{α}-(benzyloxycarbonyl)citrulline methyl ester (**1**) identical in all respects with an authentic sample.

In view of the successful transformation of ornithine to arginine coupled with established procedures[7] for the transformation of the latter to urea and ornithine, the work outlined here formally completes the chemical simulation of the salient features of the urea cycle (Figure 4).

Experimental Section

Melting points are uncorrected. Silica gel G (Merck) was used for TLC, and column chromatography was performed with silica gel (ACME, 100–200 mesh). Reactions were monitored wherever possible by TLC. The organic extracts were invariably dried over anhydrous $MgSO_4$, and solvents were evaporated in vacuo. ^1H NMR spectra were obtained at 60 and 80 MHz, respectively.

N^{α}-(Benzyloxycarbonyl)-L-citrulline methyl ester (1) was prepared from N^{α}-Z-L-citrulline[8] (9.5 g, 0.03 mol) by esterification with diazomethane. Crystallization from ethyl acetate afforded 8.2 g (82%) of **1** as colorless crystals: mp 155–56 °C (lit.[9] mp 155–57 °C); IR 3460, 3320, 3280, 1740, 1630, 1535 cm^{-1}; NMR ($CDCl_3$ + DMSO-d_6) δ 1.61 (br, 4 H), 3.05 (m, 2 H), 3.65 (s, 3 H), 4.15 (m, 1 H), 5.05 (s, 2 H), 5.22 (br, 2 H), 5.90 (br, 1 H), 7.32 (s, 6 H); MS m/z 323 (M^+). Anal. Calcd for $C_{15}H_{21}N_3O_5$: C, 55.72; H, 6.50; N, 13.00. Found: C, 55.68; H, 6.64; N, 12.56.

N^{α}-Benzoyl-L-citrulline methyl ester (5) was prepared from N^{α}-Bz-L-citrulline[10] (1.7 g, 0.006 mol) by passing HCl gas through a stirred suspension in methanol (20 mL) until saturation. The resulting clear solution was evaporated in vacuo, the oily residue was suspended in distilled water, adjusted to pH 6 with 2 N NaOH, and filtered, and the residue was washed with cold water and crystallized from aqueous methanol to give 1.5 g (84%) of **5**: $[\alpha]^{26}_D$ = −12.97° (*c* 3.36, MeOH); mp 149 °C (lit.[10] mp 146–48 °C); IR 3500, 3380, 3260, 1760, 1650, 1615, 1560 cm^{-1}; NMR ($CDCl_3$ + DMSO-d_6) δ 1.78 (m, 4 H), 3.15 (m, 2 H), 3.75 (s, 3 H), 4.51 (m, 1 H), 7.35–8.35 (m, 5 H), 8.70 (d, 1 H, J = 7.5 Hz); MS m/z 275 (M − NH_4^+). Anal. Calcd for $C_{14}H_{19}N_3O_4$: C, 57.33; H, 6.48; N, 14.33. Found: C, 57.20; H, 6.65; N, 14.10.

N^{α}-Benzoyl-L-citrulline ethyl ester (8) was prepared in a similar manner as **5** by the esterification of N^{α}-Bz-L-citrulline (2 g, 0.007 mol) with ethanolic HCl. Crystallization from aqueous ethanol yielded 1.2 g (55%) of **8** as colorless needles: mp 109–10 °C; IR 3470, 3375, 3310, 3290, 1735, 1660, 1635, 1600, 1530 cm^{-1}; NMR ($CDCl_3$ + DMSO-d_6) δ 1.25 (t, J = 7.2 Hz, 3 H), 1.70 (m, 4 H), 3.12 (m, 2 H), 3.87–4.74 (m, 3 H), 7.10–8.00 (m, 5 H), 8.29–8.58 (m, 1 H); MS m/z 290 (M^+ − NH_3). Anal. Calcd for $C_{15}H_{21}N_3O_4$: C, 58.63; H, 6.84; N, 13.68. Found: C, 58.25; H, 6.85; N, 13.34.

N^{α}-(Benzyloxycarbonyl)-N^{δ}-cyanoornithine Methyl Ester (2). To an ice-cooled and stirred solution of **1** (4.20 g, 0.013 mol) in dry pyridine (40 mL) was added in three portions *p*-toluenesulfonyl chloride (7.43 g, 0.039 mol). The reaction mixture was stirred for 3 h at room temperature, poured on to ice (~400 g), extracted with ethyl acetate (3 × 100 mL), washed with water (3 × 100 mL), 2 N H_2SO_4 (2 × 50 mL), and water (3 × 100 mL), dried, and evaporated in vacuo to give 3.96 g (quantitative yield) of cyanoornithine **2** as a viscous oil: IR 3320, 2215, 1730–1650 (br), 1515 cm^{-1}; NMR ($CDCl_3$) δ 1.76 (br, 4 H), 3.06 (m, 2 H), 3.77 (s, 3 H), 4.29 (br, 1 H), 4.64 (br, 1 H), 5.15 (s, 2 H), 5.75 (d, J = 9 Hz, 1 H), 7.39 (s, 5 H); MS m/z 305 (M^+). Anal. Calcd for $C_{15}H_{19}N_3O_4$: C, 59.01; H, 6.22; N, 13.77. Found: C, 58.60; H, 6.15; N, 13.35.

N^{α}-Benzoyl-N^{δ}-cyanoornithine methyl ester (6) was prepared in a similar manner as **2** from **5** (1.17 g, 0.004 mol) and *p*-toluenesulfonyl chloride (2.28 g, 0.012 mol) in pyridine (8 mL). The product on crystallization from EtOAc–hexane gave 0.61 g (55%) of cyanoornithine **6** as colorless prisms: $[\alpha]^{26}_D$ = +36.48° (*c* 3.36, $CHCl_3$); mp 75 °C; IR 3310, 2240, 1745, 1645, 1540 cm^{-1}; NMR ($CDCl_3$) δ 1.73 (br, 4 H), 2.93 (br, 2 H), 3.61 (s, 3 H), 4.60 (br, 1 H), 5.13 (br, 1 H), 6.97–7.96 (m, 6 H); MS m/z 275 (M^+). Anal. Calcd for $C_{14}H_{17}N_3O_3$: C, 61.09; H, 6.18; N, 15.27. Found: C, 60.95; H, 6.35; N, 15.09.

N^{α}-Benzoyl-N^{δ}-cyanoornithine ethyl ester (9), prepared from **8** (0.3 g, 0.001 mol), *p*-toluenesulfonyl chloride (0.57 g, 0.003 mol), and pyridine (3 mL) in a similar manner as **2** and **6**, was crystallized from EtOAc–hexane as colorless needles: yield 0.16 g (56%); mp 85 °C; IR 3310, 2220, 1745, 1655, 1550 cm^{-1}; NMR ($CDCl_3$) δ 1.28 (t, J = 7 Hz, 3 H), 1.82 (m, 4 H), 3.12 (m, 2 H), 4.22 (q, J = 7 Hz, 2 H), 4.73 (m, 2 H), 6.97 (d, J = 7 Hz, 1 H), 7.13–7.84 (m, 5 H); MS m/z 289 (M^+). Anal. Calcd for $C_{15}H_{19}N_3O_3$: C, 62.28; H, 6.57; N, 14.53. Found: C, 61.79; H, 6.59; N, 14.31.

[N^{α}-(Benzyloxycarbonyl)arginino]succinic Acid Trimethyl Ester (4), the Imidazolone 11, and the Imidazolinone Imine 12. To a solution of dimethyl aspartate hydrochloride (0.591 g, 0.003 mol) in distilled water (7 mL), adjusted to pH 3.7 by addition of saturated $NaHCO_3$, was added a solution of cyanoornithine **2** (0.915 g, 0.003 mol) in acetonitrile (8 mL). The reaction mixture was refluxed for 10 h and cooled, the solvents were evaporated in vacuo, and the residue chromatographed over a column of silica gel. Elution with a mixture of EtOAc–MeOH, 98:2, yielded the unreacted **2** (0.216 g, 24%), with EtOAc–MeOH, 88:12; Z-Cit-OMe (0.230 g, 24%), with EtOAc–MeOH, 85:15; imidazolone **11** (0.018 g, 1%) as viscous oil followed by imidazolinone imine **12** (0.061 g, 5%) as gummy solid; and finally the argininosuccinate **4** (0.555 g, 40%) as foamy solid.

4: foamy, cream-colored hygroscopic substance; IR 3600–3000 (br), 1760–1700 (br), 1630, 1530 cm^{-1}; NMR ($CDCl_3$) δ 1.88 (br, 4 H), 3.09 (br, 2 H), 3.50 (br, 2 H), 3.78 (m, 9 H), 4.39 (br, 2 H), 5.12 (s, 2 H), 6.32 (br, 1 H), 7.12 (br, 3 H), 7.41 (s, 5 H); MS m/z 466 (M^+), 448 (M − NH_4^+), 389 (M − (NH_4^+ + COOMe)). Anal. Calcd for $C_{21}H_{30}N_4O_8$: C, 54.07; H, 6.43; N, 12.01. Found: C, 54.14; H, 6.26; N, 12.22. **11**: NMR ($CDCl_3$) δ 1.83 (br, 4 H), 3.00 (br, 2 H), 3.47 (br, 2 H), 3.77 (s, 6 H), 4.35 (br, 2 H), 5.12 (s, 2 H), 6.00 (br, 1 H), 7.40 (s, 7 H); MS m/z 434 (M^+), 417 (M^+ − NH_3), 402 (M^+ − MeOH). **12**: IR 3400 (br), 1700 (br), 1620, 1520 cm^{-1}; NMR ($CDCl_3$) δ 1.85 (br, 4 H), 2.95 (br, 2 H), 3.43 (br, 2 H), 3.63 (s, 3 H), 3.75 (s, 3 H), 4.31 (br, 2 H), 5.12 (s, 2 H), 6.19 (br, 1 H), 7.35 (s, 7 H); MS m/z 416 (M − NH_4^+), 402 (M^+ − MeOH).

(N^{α}-Benzoylarginino)succinic acid trimethyl ester (7) was prepared under conditions described above from **6** (0.412 g, 0.0015 mol) and dimethyl aspartate hydrochloride (0.295 g, 0.0015 mol) in aqueous acetonitrile at pH 3.7 under reflux for 10 h. The crude reaction mixture when subjected to column chromatography on silica gel gave, on elution with EtOAc–PhH, 80:20, **6** (0.124 g, 30%); with EtOAc–MeOH, 95:5, **5** (0.07 g, 16%); and with EtOAc–MeOH, 90:10, the desired adduct **7** (0.39 g, 60%): foamy solid; mp 85–100 °C; IR 3600–3000 (br), 1740, 1700, 1635, 1535 cm^{-1}; NMR ($CDCl_3$) δ 1.87 (br, 4 H), 3.00 (br, 2 H), 3.59 (br, 2 H), 3.77 (m, 9 H), 4.65 (br, 2 H), 7.00–8.00 (m, 9 H); MS m/z 418 (M − NH_4^+). Anal. Calcd for $C_{20}H_{28}N_4O_7$: C, 55.04; H, 6.42; N, 12.84. Found: C, 55.33; H, 6.87; N, 13.36.

(N^{α}-Benzoylarginino)succinic acid α-ethyl ω,ω'-dimethyl ester (10) was obtained by reaction of cyanoornithine **9** (1.73 g, 0.006 mol) and dimethyl aspartate hydrochloride (1.18 g, 0.006 mol) at pH 3.7 under conditions described above: yield 1.48 g (55%); viscous oil; IR 3400, 3040, 1735, 1650, 1530 cm^{-1}; NMR ($CDCl_3$ + DMSO-d_6) δ 1.35 (t, J = 7.5 Hz, 3 H), 1.88 (br, 4 H), 2.85 (m, 2 H), 3.74 (m, 8 H), 4.25 (q, J = 7.5 Hz, 2 H), 4.65 (m, 2 H), 7.35–8.15 (m, 5 H), 8.75 (d, J = 7.7 Hz, 1 H); MS m/z 432 (M − NH_4^+), 418 (M^+ − MeOH), 373 (M − (NH_4^+ + COOMe)), 359 (M − (NH_4^+ + COOEt)).

Dimethyl N^{α}-(4-Quinazolyl)aspartate (15). (a) Dimethyl Aspartate (3). To an ice-cooled and stirred suspension of dimethyl aspartate hydrochloride (3.71 g, 0.019 mol) in dry CH_2Cl_2 (25 mL) was added in drops triethylamine (1.9 g, 0.019 mol). The reaction mixture was stirred for 0.5 h, poured on to crushed ice (~100 g), extracted with CH_2Cl_2 (2 × 50 mL), dried, and evaporated to give 2.3 g (76%) of dimethyl aspartate **3** as an oil, which was used without further delay: NMR ($CDCl_3$) δ 1.87 (br, 2 H), 2.65 (dd, J = 6, 0.5 Hz, 2 H), 3.59 (s, 3 H), 3.65 (s, 3 H).

(b) Reaction of 3 with 4-Chloroquinazoline. A stirred solution of 4-chloroquinazoline[11] (0.228 g, 0.0014 mol) in dry xylene (5 mL) was admixed with a solution of freshly prepared **3** (0.56

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g, 0.0035 mol) in dry xylene (2 mL). The reaction mixture was refluxed for 3 h and cooled, the solvents were evaporated in vacuo, and the residue was chromatographed on silica gel. Elution with PhH–EtOAc, 40:60, gave 0.094 g (24%) of **15** as pale yellow solid, mp 176 °C. The yield is based on actually isolated material; TLC showed the presence of unchanged 4-chloroquinazoline, which was not isolated: IR 3400 (br), 3180, 1720, 1600, 1565 cm^{-1}; NMR ($CDCl_3$) δ 3.15 (d, J = 5 Hz, 2 H), 3.70 (s, 3 H), 3.80 (s, 3 H), 5.37 (br, 1 H), 7.05 (br, 1 H), 7.15–7.95 (m, 4 H), 8.60 (s, 1 H); MS m/z 289 (M^+), 230 (M^+ - COOMe), 144 (dimethyl fumarate)$^+$, 129 (quinazoline)$^+$. Anal. Calcd for $C_{14}H_{15}N_3O_4$: C, 58.13; H, 5.19; N, 14.53. Found: C, 57.92; H, 5.48; N, 14.28.

Dimethyl N^α-(9-Benzyl-6-purinyl)aspartate (18). To a solution of 9-benzyl-6-chloropurine[12] (0.245 g, 0.001 mol) in dry xylene (5 mL) was added in drops a solution of freshly prepared **3** (0.403 g, 0.0025 mol) in dry xylene (2 mL). The reaction mixture was refluxed for 8 h, cooled, and decanted, the residue was triturated with dry xylene (2 mL), the combined organic extracts were evaporated, and the residue was chromatographed on silica gel. Elution with PhH–EtOAc, 50:50, gave 0.093 g (37%) of unchanged 9-benzyl-6-chloropurine and with PhH–EtOAc, 30:70, gave a 0.155 g (42%) of the adduct **18** as a viscous oil: IR 3550 (br), 3350 (br), 1730, 1600, 1570 cm^{-1}; NMR ($CDCl_3$) δ 3.00 (d, J = 5 Hz, 2 H), 3.60 (s, 3 H), 3.66 (s, 3 H), 5.26 (s, 2 H), 6.53 (br, 1 H), 7.16 (s, 5 H), 7.60 (s, 1 H), 8.26 (s, 1 H); MS m/z 369 (M^+), 310 (M^+ - COOMe), 278 (M^+ - CH_2Ph). Anal. Calcd for $C_{18}H_{19}N_5O_4$: C, 58.53; H, 5.14; N, 18.97. Found: C, 58.62; H, 4.98; N, 18.45.

Demonstration of the Thermal Cleavage of Dimethyl N^α-(4-Quinazolyl)aspartate (15) in the Presence of Butyric Acid: Isolation of 4-Aminoquinazoline (16). A solution of **15** (0.020 g, 0.00007 mol) in dry xylene (2 mL) was admixed with butyric acid (0.1 mL), refluxed for 6 h, and cooled, the solvents were evaporated, and the residue was admixed with water (5 mL) followed by aqueous ammonia to effect neutralization; the mixture was then extracted with hot EtOAc (2 × 20 mL), dried, and evaporated to give 0.005 g (50%) of 4-aminoquinazoline (**16**), mp 265 °C, which was found identical in all respects with that of an authentic sample (lit.[13] mp 266 °C): IR 3400 (br), 3360, 3260, 3080, 1670, 1600, 1570 cm^{-1}.

Demonstration of the Cleavage of Dimethyl N^α-(9-Benzyl-6-purinyl)aspartate (18) with Butyric Acid: Isolation of 9-Benzyladenine (19). A stirred solution of the adduct **18** (0.155 g, 0.00042 mol) and butyric acid (1 mL) was refluxed for 10 h, cooled, admixed with water (10 mL), neutralized with aqueous ammonia to pH 8, extracted with hot EtOAc (3 × 20 mL), dried, and evaporated, and the residue on preparative TLC, using 100% EtOAc as a developer, gave 0.04 g (11%) of unchanged **18** and 0.04 g (42%) of 9-benzyladenine (**19**), mp 234 °C, which was found identical in all respects with that of an authentic sample (lit.[14] mp 235 °C): IR 3400 (br), 3290, 1635, 1590 cm^{-1}; NMR (DMSO-d_6) δ 5.41 (s, 2 H), 7.30 (m, 5 H), 8.22, 8.31 (s, s, 1 H, 1 H).

Reaction of N^α-Benzoyl-N^δ-cyanoornithine Methyl Ester (6) with Dimethyl Aspartate Hydrochloride at pH 2.5: Isolation of N^α-Bz-Cit-OMe (5), Argininosuccinate 7, and N^α-Bz-Arg-OMe Hydrochloride (13). To a solution of dimethyl aspartate hydrochloride (0.295 g, 0.0015 mol) in distilled water (6 mL), adjusted to pH 2.5 by addition of saturated $NaHCO_3$, was added a solution of cyanoornithine **6** (0.412 g, 0.0015 mol) in acetonitrile (7 mL). The reaction mixture was refluxed for 40 h and cooled, the solvents were evaporated in vacuo, and the residue was chromatographed over silica gel and eluted with EtOAc–MeOH, 95:5, to give **5** (0.127 g, 29%); with EtOAc–MeOH, 90:10, to give the argininosuccinate **7** (0.196 g, 30%); and finally with EtOAc–MeOH, 88:12, to give the cleaved product, N^α-Bz-Arg-OMe hydrochloride (**13**) (0.093 g, 19%), which was found to be identical in all respects with an authentic sample prepared by two methods.[15-17]

Cleavage of (N^α-Benzoylarginino)succinic Acid Trimethyl Ester (7) to N^α-Benzoylarginine Methyl Ester Hydrochloride (13). A stirred solution of **7** (0.140 g, 0.0003 mol) in acetonitrile (5 mL) was admixed with 5 mL of 0.03 N HCl, adjusted to pH 2.5 by addition of few drops of saturated $NaHCO_3$, and refluxed for 40 h, the solvents were evaporated in vacuo, and the residue was chromatographed over a column of silica gel. Elution with EtOAc–MeOH, 85:15, afforded 0.059 g (56%) of **13**, mp 86–88 °C, which was found identical in all respects with an authentic sample.

N^α-Benzoyl-L-arginine Methyl Ester Hydrochloride. Procedure A. L-Arginine monohydrochloride (1.96 g, 0.01 mol) was benzoylated specifically in saturated sodium bicarbonate, and the resulting N^α-benzoyl-L-arginine[15] was esterified via methanolic HCl to afford N^α-benzoylarginine methyl ester hydrochloride (1.9 g, 60% overall yield), mp 87–89 °C (MeOH–Et_2O).

Procedure B. L-Arginine monohydrochloride (0.98 g, 0.005 mol) was transformed to N^α-benzoylarginine methyl ester bicarbonate,[16,17] dissolved in dry ethereal HCl, and evaporated in vacuo to give N^α-benzoylarginine methyl ester hydrochloride (0.47 g, 30% overall yield: $[\alpha]^{26}_D$ = −19.8° (c 3.33, MeOH); mp 86–89 °C; IR 3365, 3205, 1740, 1645, 1580, 1535, 1490, 1470, 1445, 1360, 1315, 1220, 1160, 720, 695 cm^{-1}; NMR (DMSO-d_6) δ 1.85 (m, 4 H), 3.05 (m, 2 H), 3.72 (s, 3 H), 4.56 (m, 1 H), 7.18–8.43 (m, 9 H), 8.75 (d, J = 8.7 Hz, 1 H). Anal. Calcd for $C_{14}H_{21}N_4O_3Cl$: C, 51.14; H, 6.39; N, 17.04. Found: C, 51.47; H, 6.83; N, 16.57.

Reaction of L-Ornithine Cu Complex with Nitrourea: Preparation of N^α-Z-Cit-OMe (1). A refluxing solution of L-ornithine hydrochloride (5.0 g, 0.03 mol) in 35 mL of water was admixed, in portions, with basic cupric carbonate (5.4 g, 0.023 mol). Refluxing was continued for 0.75 h. The reaction mixture was filtered while hot and washed with water (5 mL). The filtrates were concentrated to half the volume in vacuo, admixed with nitrourea[18] (10 g, 0.095 mol), refluxed for 30 h, cooled, filtered, and adjusted to pH 5 with 2 N HCl. The copper complex was decomposed with H_2S, filtered, evaporated in vacuo to dryness, triturated with EtOAc (50 mL), filtered, and evaporated. The residue was dissolved in ice-cooled 1 N NaOH (18 mL) and subjected to simultaneous addition of Z-Cl (3.5 mL) and 1 N NaOH, keeping the medium basic throughout. The reaction mixture was stirred at room temperature for 0.5 h and extracted with ether (2 × 50 mL), and the aqueous layer was adjusted to pH 2 with 5 N HCl, extracted with EtOAc (2 × 100 mL), dried, and evaporated to give 1.65 g (overall yield 18%) of N^α-Z-Cit, which was quantitatively esterified with diazomethane to afford N^α-Z-Cit-OMe (**1**), mp 153–155 °C, identical with that of an authentic sample.

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Registry No. **1**, 89499-13-8; **1** free acid, 6692-89-3; **2**, 125735-05-9; **3**, 6384-18-5; **3**·HCl, 32213-95-9; **4**, 125735-06-0; **5**, 14325-36-1; **6**, 125735-07-1; **7**, 125735-08-2; **8**, 47235-26-7; **9**, 28908-04-5; **10**, 125735-09-3; **11**, 125735-10-6; **12**, 125735-11-7; **13**, 1784-04-9; **13** bicarbonate, 125735-14-0; **15**, 125735-12-8; **16**, 15018-66-3; **18**, 125735-13-9; **19**, 4261-14-7; Z-Cl, 501-53-1; 4-chloroquinazoline, 5190-68-1; 9-benzyl-6-chloropurine, 1928-76-3; L-ornithine hydrochloride, 20724-48-5; nitrourea, 556-89-8; arginine, 74-79-3; urea, 57-13-6.

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