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The Total Large-Scale Synthesis of Argiopine

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Abstract—The total large-scale synthesis of a natural toxin argiopine, a polymethylenepolyamine derivative, was developed. It consisted of 26 stages and included three key block schemes. Most of the stages proceeded quantitatively, which excluded the necessity of using the chromatographic separation of intermediates.

Key words: argiopine, polymethylenepolyamines, synthesis

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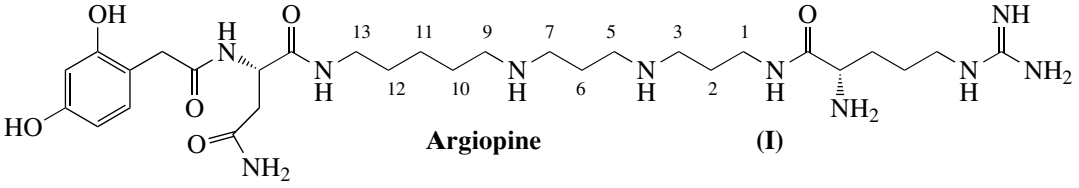
INTRODUCTION

Argiopine (argiotoxin, M 636) (**I**) is a toxin of the *Argiope lobata* spider venom. It was the first acylpolyamine whose structure had been established by chemical methods, mass spectrometry, and NMR spectroscopy [1, 2]. Its basis is a polyamine 1,13-diamino-4,8-diazatridecane, $NH_2(CH_2)_5NH(CH_2)_3NH(CH_2)_3NH_2$, acylated by an *L*-arginine residue at N1 and by an *L*-asparagine residue at N13; in turn, the Asp is *N*-acylated by 2,4-dihydroxyphenylacetic acid.



Argiopine **(I)**

The structure of argiopine was confirmed by countersynthesis [3–6]. However, the methods described included stages leading to target products in low yields or requiring the use of difficult-to-find starting compounds. The authors widely applied chromatography for the purification of reaction products; therefore, their synthetic schemes were suitable for obtaining only small quantities of the target product.

We had the task of obtaining argiopine on the large scale. Hence, we had to develop synthetic methods for each fragment with a minimally possible number of stages; each of them should not practically lead to side products, provide yields of no less than 85%, and possibly exclude the chromatographic purification of the products of synthesis. When possible, we used the methods developed in the above works, especially those described in [6].

RESULTS AND DISCUSSION

The analysis of the argiopine structure suggests that the most logical way of synthesis consists of the convergent buildup of the target molecule. A retrosynthetic analysis allowed us to develop the following synthetic schemes that meet the conditions (Schemes 1–4).

Obtaining 2,4-dihydroxyphenylacetic acid. A fragment of 2,4-dihydroxyphenylacetic acid bound with an asparagine N^{α} atom was introduced [3–6] using an

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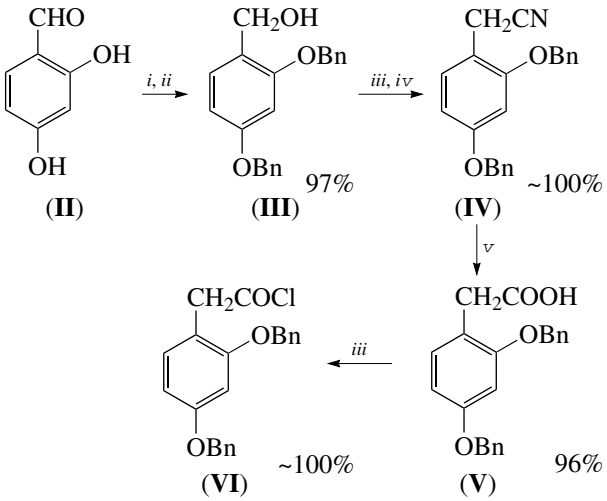
active (*N*-succinimide or *p*-nitrophenylphenyl) ester of 2,4-dibenzyloxyphenylacetic acid. Benzyl ethers as the protection of phenol hydroxy groups are stable under condensation conditions with an asparagine fragment and are easily removable by hydrogenolysis. At the same time, the active esters used are improper for large-scale synthesis because *N*-hydroxysuccinimide or *p*-nitrophenol arising as the result of *N*-acylation are difficult to remove from the reaction mixtures, which leads to inevitable losses and a decrease in the yield of the target compound. Therefore, we chose a 2,4-dibenzyloxyphenylacetic acid chloride that is sufficiently active in the condensation reaction with primary amine; the evolving HCl can easily be bound with triethylamine.

2,4-Dibenzyloxyphenylacetic chloride (**VI**) (Scheme 1) was obtained in a general yield of 83% from 2,4-dihydroxybenzaldehyde (**II**). This compound was treated with a small excess of benzyl chloride in DMF in the presence of K_2CO_3, and the aldehyde group was reduced by sodium borohydride in an ethanol–propane-2-ol mixture; the yield of (**III**) was 97%. The hydroxyl group was then exchanged with halogen by the treatment of primary alcohol with thionyl chloride in dry benzene; the subsequent treatment with sodium cyanide in DMSO led to a quantitative yield of 2,4-dibenzyloxyphenylacetonitrile (**IV**). The nitrile group was easily hydrolyzed by alkali to a carboxyl group (compound (**V**), yield 96%); the treatment of the resulting substituted phenylacetic acid with thionyl chloride in dry benzene almost quantitatively led to the target chloride **VI**.

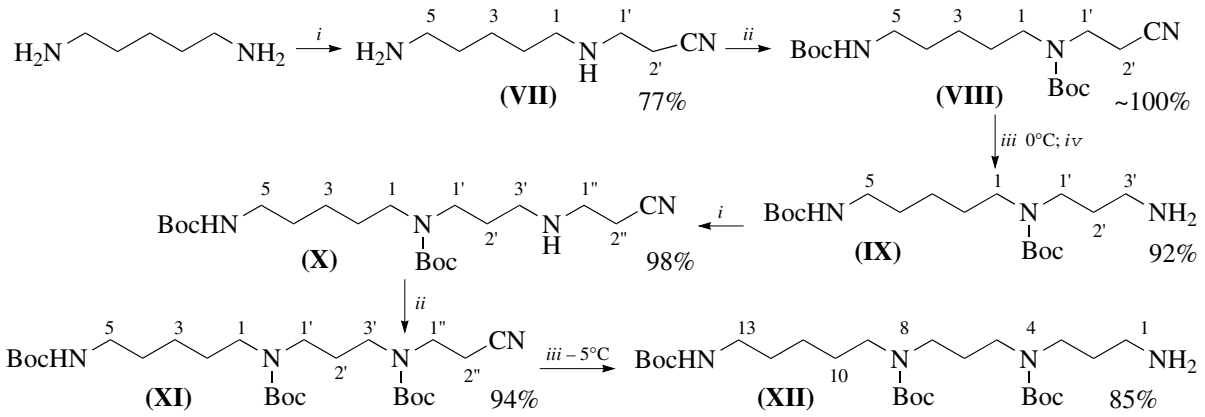


Reagents: *i* – BnCl, DMF, K_2CO_3; *ii* – $NaBH_4$, EtOH/Me_2CHOH; *iii* – $SOCl_2$, DMF/PhH; *iv* – NaCN, DMSO; *v* – NaOH, H_2O/EtOH.

Scheme 1.

Obtaining the polyamine fragment. The polyamine fragment of the argiopine structure (**I**), –$NH(CH_2)_5NH(CH_2)_3NH(CH_2)_3NH$–, can be obtained by a number of pathways. Yelin et al. [4] used the condensation of 1,5-diaminopentane (cadaverine) with *N*-trityl-β-alanyl-β-alanine followed by the reduction of oxo groups to methylene groups by lithium aluminum hydride. However, this method is complex and the polyamine was isolated in a low yield. A twofold alkylation of protected cadaverine with phthalimidopropyl bromide [5] also did not provide high yields of the target products. The condensation of *N*-monoprotected (Boc or Z) cadaverine with acrylonitrile, the reduction of nitrile to the aminomethyl group, and the repetition of this process with the intermediate introduction of the protective Boc group in the amino group were used in [3, 6].

This way appears to be the most convenient. However, we changed the sequence of stages (Scheme 2). The coupling of a twofold molar excess of cadaverine with acrylonitrile in methanol during cooling proceeded with the formation of both mono- and bis-cyanoethylation products. Vacuum distillation helped us isolate the unreacted cadaverine (bp 68–72°C/12 mm Hg) and 1-(2-cyanoethyl)-1,5-diaminopentane (**VII**) (bp 108–110°C/0.2 mm Hg), yield 77%.

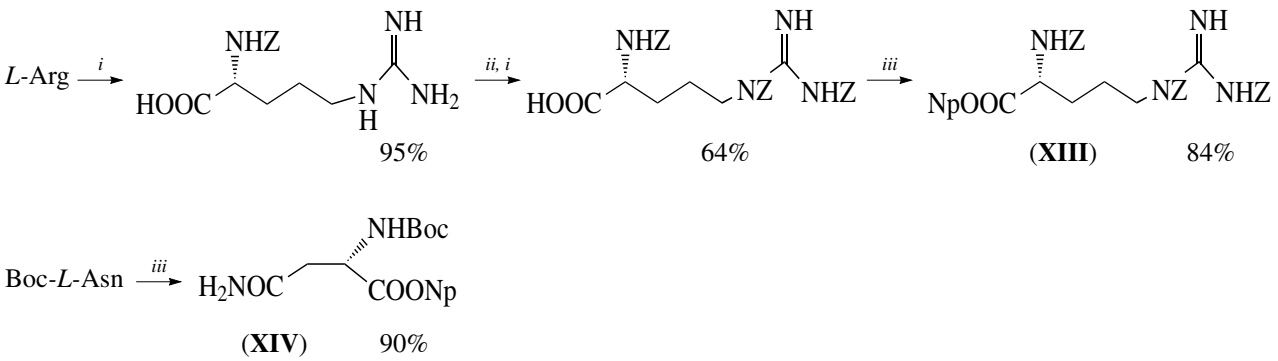


Reagents: *i* – CH_2=CHCN, MeOH, 0°C; *ii* – Boc_2O, CH_2Cl_2, room temperature; *iii* – $LiAlH_4$, Et_2O; *iv* – NaOH/H_2O.
(The carbon atom numbering in NMR spectra is given)

Scheme 2.

The introduction of two *N*-protective Boc groups proceeded easily, and compound (**VIII**) was isolated in a quantitative yield. The reduction of the nitrile group to the aminomethyl group was carried out by lithium aluminum hydride at 0°C in dry diethyl ether. The treatment of the reaction mixture with alkali allowed for the separation of lithium and aluminum salts by simple filtration and the isolation of (**IX**) in a 92% yield. The chemical shifts, multiplicities, and intensities of signals in the ^{1}H NMR spectrum of (**IX**) confirmed its structure as 1-(3-aminopropyl)-1,5-di-Boc-aminopentane. Further elongation of the chain was carried out by the repetition of the above-described sequence of operations: the coupling of (**IX**) with acrylonitrile in methanol in a practically quantitative yield, the introduction of an *N*-protective Boc group into the amino group, and the reduction of the nitrile group to the aminomethyl group by lithium aluminum hydride in ether. The chemical shifts, multiplicities, and intensities of the proton signals in the ^{1}H NMR spectrum of (**XII**) completely correspond to its structure. The total yield of this derivative after seven synthetic steps was 56% from cadaverine.

Synthesis of amino-acid fragments. Tri-Z-*L*-arginine *p*-nitrophenyl ester (**III**) was obtained by a step introduction of Z groups into *L*-arginine (Scheme 3).



Reagents: *i* – ZCl, $NaHCO_3$; *ii* – Me_3SiCl; $(Pr^i)_2EtN$; *iii* – NpOH, DCC, DMF.

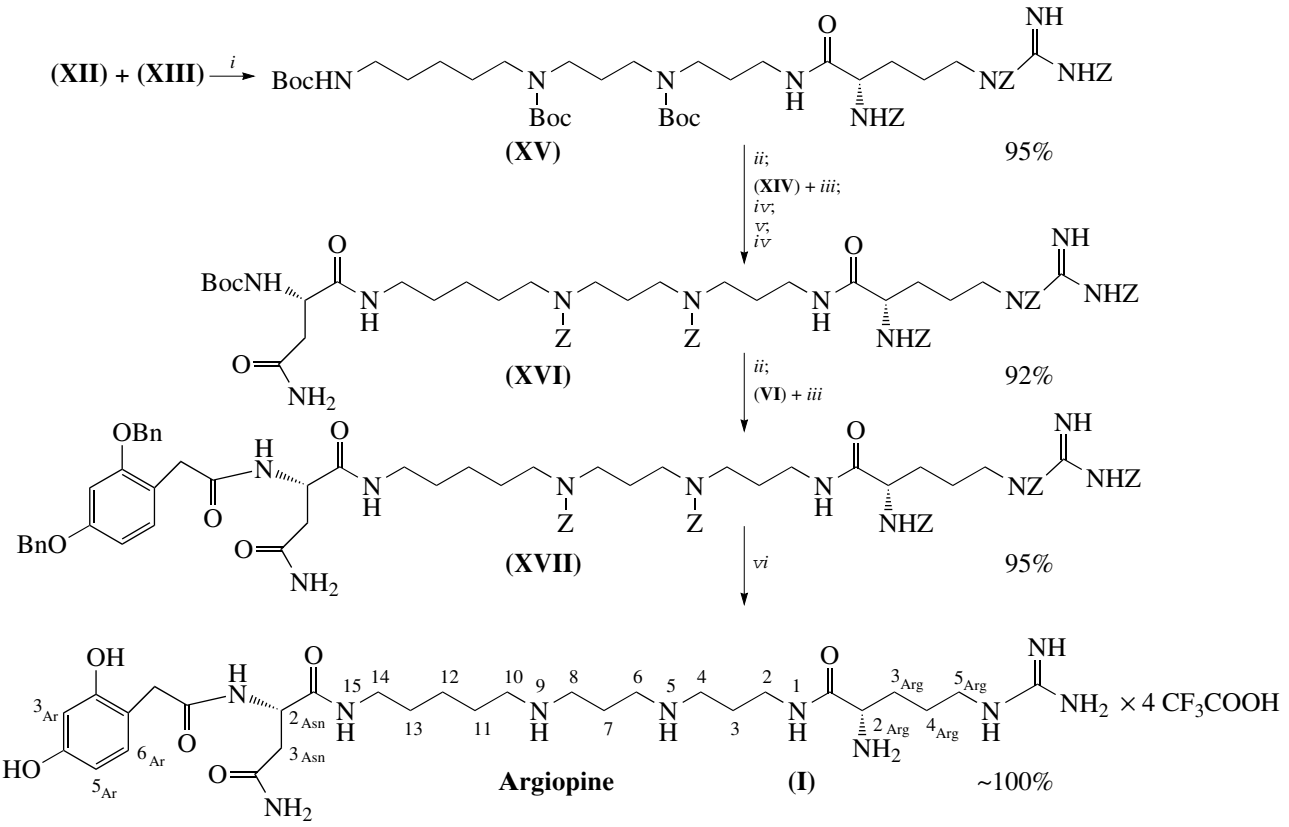
Scheme 3.

First, we protected the α-amino group, then we introduced the *N*-trimethylsilyl protective groups and the intermediate N^{α}-Z-δ,ω-bis-Me_3Si-*L*-arginine treated with Z-Cl; the yield of tri-Z-*L*-arginine was 64%. Tri-Z-*L*-arginine *p*-nitrophenyl ester (**XIII**) was obtained by the known method in a general yield of 51%. The physicochemical properties of the resulting compound coincided with the published data, and the

mp and the value of $[\alpha]_D$ confirmed the retention of the *L*-configuration of the amino acid. A similar derivative was used in [3, 5].

Boc-*L*-asparagine *p*-nitrophenyl ester (**XIV**) was obtained in a 90% yield by the treatment of Boc-*L*-asparagine with *p*-nitrophenol in the presence of DCC; the $[\alpha]_D$ value of the resulting compound indicated the retention of the amino acid *L*-configuration.

*Synthesis of argiopine (**I**).* The interaction of (**XII**) containing a free amino group in position 1 with the tri-Z-*L*-arginine was carried out in THF at room temperature; (**XV**) was obtained in a 95% yield (Scheme 4). The subsequent three stages—(a) the removal of three *N*-protective Boc groups, (b) the selective acylation of the primary amino group by Boc-*L*-asparagine active ester (**XIV**), and (c) the introduction of two benzyloxycarbonyl groups in the secondary amino groups of the polyamine fragment—were carried out without the isolation of intermediates. This lucky finding allowed us to avoid the usual losses at the stages of isolation and purification of the intermediates. The search for the conditions of these reactions and the separation of products without any chromatographic purification required a laborious investigation, which led to the isolation of (**XVI**) in a 92% yield.



Reagents: *i* – THF, 24 h, room temperature; *ii* – CF_3COOH, CH_2Cl_2; *iii* – THF, Et_3N; *iv* – 12 h, room temperature; *v* – BnOCOCl, DMF, Et_3N, 0°C; *vi* – H_2, 10% Pd/C, MeOH, CF_3COOH, room temperature.

Scheme 4.

The residue of dibenzyloxyphenylacetic acid was introduced into (**XVI**) after the removal of its *N*-protective Boc group from the asparagine residue by trifluoroacetic acid and then, without isolation of the intermediate, by the acylation of the free α-amino group of asparagine with chloride (**VI**) in THF in the presence of Et_3N at 0°C (Scheme 4).

Compound (**XVII**) was purified by recrystallization; its yield was 95%. The chemical shifts, multiplicities, and intensities of all of the proton signals in the ^1H NMR spectrum of (**XVII**) completely correspond to its structure.

The obtained compound (**XVII**) contains two types of protective groups: benzyl and benzyloxycarbonyl.

We succeeded in finding conditions for the simultaneous removal of two types of groups. The hydrogenolysis of (**XVII**) in the presence of Pd/C and trifluoroacetic acid led to a quantitative yield of pure argiopine (**I**) in the form of a salt with four molecules of trifluoroacetic acid.

According to the spectral characteristics (^{1}H NMR and mass spectra), the resulting argiopine coincided with the natural sample.

The large-scale method of argiopine synthesis we suggested differs from the previously described methods [3–6] in the practically unambiguous passage of all of the reactions and the high yields of the target compounds, which allowed us to use them without any additional purification. We succeeded in finding the conditions for several successive stages without the isolation of intermediates. Thus, we prepared 130 g of argiopine in the form of tetrakis(trifluoroacetate), a synthetic scheme including 26 stages.

EXPERIMENTAL

^{1}H NMR spectra (δ, ppm, *J*, Hz) were registered on a Varian Unity INOVA spectrometer (United States) with a working frequency of 400 MHz in $CDCl_3$ relative to the residual protons of the solvent (δ_H 7.25 ppm). The spectrum of argiopine (**I**) was measured in CD_3OD with tetramethylsilane as an internal standard. Optical activity was determined on a DIP-360 polarimeter (Japan). Mass spectra were registered on a Finnigan Mat 8430 spectrometer (Thermo Electron, Germany) at the electron impact ionization. The argiopine (**I**) mass spectrum was obtained on a MALDI–TOF mass spectrometer (Vision 2000, United States). Melting points were determined on a Boetius microtable.

TLC was carried out on Kieselgel F-60 plates (Merck, Germany) using the following solvent systems: A, 1%MeOH in $CHCl_3$; B, 2% MeOH in $CHCl_3$; C, 5% MeOH in $CHCl_3$; and D, 10% MeOH in $CHCl_3$. Substance spots were detected by iodine vapor.

*2,4-Dihydroxybenzaldehyde (**II**)* was obtained by the formylation of resorcinol under conditions of the Vilsmeier reaction [7]; yield 60%, mp 136°C; lit. [7] mp 135°C.

*2,4-Dibenzyloxybenzyl alcohol (**III**).* Compound (**II**) (41.4 g, 0.3 mol) was added to a stirred suspension of anhydrous K_2CO_3 (85.5 g) in DMF (150 ml). The reaction mixture was heated to 50°C and BnCl (86.4 ml, 95.0 g, 0.75 mol) was dropwise added at this temperature. Then, the reaction mixture was stirred for 2 h at 100°C, cooled, and poured into water (1500 ml). The oil separated was dissolved in a minimal quantity of chloroform and filtered through a silica gel layer. The filtrate was evaporated to dryness, and 2,4-dibenzyloxybenzaldehyde (85.8 g, 90%) was obtained in the form of a rapidly crystallizing oil, mp 85°C, ^{1}H NMR spectrum: 10.38 (1 H, s, CHO), 7.83 (1 H, d, *J* 8, H6), 7.31–7.43 (10 H, m, Ph), 6.63 (1 H, dd, *J* 2.2 and 8, H5), 6.59 (1 H, d, *J* 2.2, H3), 5.12 (2 H, s, CH_2Ph), 5.08 (2 H, s, CH_2Ph); MS, *m*/*z*: 318 (M^+); lit. [8]: mp 85–86°C.

$NaBH_4$ (5.0 g, 0.13 mol) was added in portions to a warm stirred suspension of the obtained compound (50.9 g, 0.16 mol) in a mixture of ethanol (250 ml) and isopropanol (1 l). As the reduction proceeded, the precipitate was dissolved, a homogeneous solution was formed, and a new precipitate began to form in 2 h. Benzene (500 ml) and water (2 l) were then added to the reaction mixture; the upper layer was separated, and the water layer was extracted with benzene (3 × 100 ml). The combined benzene solutions were dried by Na_2SO_4, evaporated, and (**III**) was obtained; yield 49.7 g (97%); mp 96°C, R_f 0.32 (A); ^{1}H NMR spectrum: 7.28–7.43 (10 H, m, Ph), 7.18 (1 H, d, *J* 8, H6), 6.62 (1 H, d, *J* 2, H3), 6.54 (1 H, dd, *J* 2 and 8, H5), 5.06 (2 H, s, CH_2Ph), 5.03 (2 H, s, CH_2Ph), 4.65 (2 H, s, CH_2O); MS, *m*/*z*: 320 (M^+).

*2,4-Dibenzyloxyphenylacetonitrile (**IV**).* DMF (1 ml) and $SOCl_2$ (16 ml) were added to a solution of (**III**) (40 g, 0.125 mol) in dry benzene (250 ml). The reaction mixture was stirred for 40 min at room temperature, evaporated in a vacuum at 30°C, and 2,4-dibenzyloxybenzyl chloride was obtained (43.3 g, ~100%), R_f 0.67 ($CHCl_3$), which was immediately used further without additional purification.

DMSO (200 ml) and NaCN (12.0 g, 0.245 mol) were added to the above-described chloride (43.3 g, 0.125 mol), and the reaction mixture was stirred for 20 h at room temperature. Water (800 ml) was then added, and the aqueous layer was separated by decantation. The residue was dissolved in dichloromethane (200 ml), washed with 5% NaCl (2 × 200 ml), and filtered through a Celite layer. The filtrate was evaporated to dryness and (**IV**) was obtained; yield 41.1 g (~100%); mp 94°C, R_f 0.57 ($CHCl_3$); ^{1}H NMR spectrum: 7.26–7.46 (10 H, m, Ph), 7.12 (1 H, d, *J* 8.2, H6), 6.62 (1 H, d, *J* 2.4, H3), 6.56 (1 H, dd, *J* 2.4 and 8.2, H5), 5.03 (4 H, s, CH_2Ph), 3.65 (2 H, s, CH_2CN); MS, *m*/*z*: 329 (M^+).

*2,4-Dibenzyloxyphenylacetic acid (**V**).* A mixture of nitrile (**IV**) (17.6 g, 53.5 mmol), ethanol (100 ml), and 32% aqueous NaOH (51 m) was refluxed under stirring for 17 h. Ethanol was then evaporated in a vacuum, the residue was diluted with hot water, and glacial acetic acid (67 ml) was added. The precipitate was separated, washed with water, and dried in a vacuum over NaOH. Compound (**V**) was obtained; yield 17.96 g (96%); mp 139°C, R_f 0.38 (B); ^{1}H NMR: 8.80 (1 H, bs, COOH), 7.19–7.40 (10 H, m, Ph), 7.03 (1 H, d, *J* 7.8, H6), 6.54 (1 H, d, *J* 2.4, H3), 6.47 (1 H, dd, *J* 2.4 and 7.8, H5),

4.95 (4 H, s, CH_2Ph), 3.54 (2 H, s CH_2CO); lit. [4]: yield 47%, mp 139–140.5°C.

*2,4-Dibenzyloxyphenylacetyl chloride (**VI**).* DMF (0.2 ml) and $SOCl_2$ (6.75 ml) were added to a suspension of (**V**) (16.4 g, 47.1 mmol) in dry benzene (100 ml). The reaction mixture was refluxed for 2 h and evaporated in a vacuum to give (**VI**); yield 17.27 g (~100%), which was then used without additional purification.

*1-(2-Cyanoethyl)-1.5-diaminopentane (**VII**).* Acrylonitrile (26.4 ml, 0.4 mol) was dropwise added for 30 min to a solution of 1,5-diaminopentane (81.6 g, 0.8 mol) in dry methanol (30 ml) at 0°C. The reaction mixture was stirred for 1 h under cooling and left overnight at room temperature. Using a fractional distillation, we isolated the 1,5-diaminopentane excess (43.3 g, 0.425 mol) and (**VII**); yield 44.82 g (77% from the reacted 1,5-diaminopentane); bp 108–110°C/0.2 mm Hg; R_f 0.54 ($CHCl_3$–MeOH–Pr^iNH_2, 4 : 4 : 1); ^{1}H NMR: 2.80 (2 H, t, *J* 6.7, H1'), 2.56 (2 H, t, *J* 7.0, H5), 2.51 (2 H, t, *J* 7.0, H5), 2.40 (2 H, t, *J* 6.7, H2'), 1.30–1.42 (4 H, m, H2, H4), 1.20–1.30 (2 H, m, H3), 1.06 (3 H, bs, NH); MS, *m/z*: 155 (M^+).

*1,5-Di(tert-butyloxycaronylamino)-1-(2-cyanoethyl)pentane (**VIII**).* A solution of Boc_2O (63.5 g, 292.6 mmol) in dichloromethane (50 ml) was added at room temperature for 1.5 h to a stirred solution of (**VII**) (22.4 g, 144.5 mmol) in dichloromethane (300 ml). The solution was stirred for 2.5 h and washed with a saturated solution of NaCl–$NaHCO_3$ (3 × 100 ml), dried with Na_2SO_4, and evaporated to give (**VIII**); yield 51.3 g (~100%); R_f 0.51 (B); ^{1}H NMR: 4.85 (1 H, bs, NH), 3.41 (2 H, t, *J* 6.7, H1'), 3.05 (2 H, t, 2 H, t, *J* 6.85, H1), 2.85 (2 H, m, H5), 2.35 (2 H, m, H2'), 1.27–1.37 (4 H, m, H2, H4), 1.22 (9 H, s, Boc), 1.18 (9 H, s, Boc), 1.05 (2 H, m, H3).

*1,5-Di(tert-butyloxycaronylamino)-1-(2-aminopropyl)pentane (**IX**).* A solution of (**VIII**) (71.0 g, 0.2 mol) in dry Et_2O (140 ml) was dropwise added to a cooled (–5°C) suspension of $LiAlH_4$ (27.5 g, 0.72 mol) in dry Et_2O (1.3 l). The reaction mixture was stirred at 0°C for 30 min, 2 M NaOH (92 ml) was added, and stirring was continued for 1.5 h. The precipitate was separated and washed on a filter with Et_2O. The filtrates were combined and evaporated; (**IX**) was obtained as a colorless oil; yield 66.1 g (92%); R_f 0.31 (D); ^{1}H NMR: 4.75 (1 H, bs, NH), 2.90–3.15 (6 H, m, H1, H4, H1'), 2.62 (2 H, m, H4), 1.52 (2 H, m, H2'), 1.27–1.40 (4 H, m, H2, H4), 1.31 (9 H, s, Boc), 1.29 (9 H, s, Boc), 1.15 (2 H, m, H3).

*1,5-Di(tert-butyloxycarbonylamino)-1-(3-(2-cyanoethyl)aminopropyl)pentane (**X**).* Acrylonitrile (20 ml, 0.3 mol) was added dropwise for 30 min to a solution of (**IX**) (66.1 g, 0.184 mol) in dry methanol (275 ml) at 0°C. The reaction mixture was stirred for 1 h at 0°C, left overnight at room temperature, and evaporated in a vacuum to give (**X**) as a colorless oil; yield 74.3 g (98%); R_f 0.28 (B); ^{1}H NMR: 4.56 (1 H, bs, BocNH), 3.37 (2 H, t, *J* 6.4, H1''), 3.17 (2 H, t, *J* 6.4, H2''), 2.92–3.15 (6 H, m, H1, H5, H1'), 2.56 (2 H, m, H3'), 1.66 (2 H, m, H2'), 1.27–1.40 (4 H, m, H2, H4), 1.32–1.38 (18 H, m, Boc), 1.19 (2 H, m, H3).

*1,5-Di(tert-butyloxycarbonylamino)-1-(3-tertbutyloxycarbonyl)-2-cyanoethyl)aminopropylpentane (**XI**).* A solution of Boc_2O (46.8 g, 214.6 mmol) in dichloromethane (80 ml) was added dropwise to a solution of (**X**) (50.54 g, 195.5 mmol) in dichloromethane (230 ml) for 30 min, and the mixture was stirred for 2.5 h at room temperature. The solution was then washed with a saturated NaCl/$NaHCO_3$ solution (3 × 100 ml), dried with Na_2SO_4, and evaporated to give (**XI**); yield 94.17 (94%) as a colorless oil; ^{1}H NMR: 4.56 (1 H, bs, BocNH), 3.37 (2 H, t, *J* 6.4, H1''), 3.17 (2 H, t, *J* 6.4, H2''), 2.92–3.15 (6 H, m, H1, H5, H1'), 2.56 (2 H, m, H3'), 1.66 (2 H, m, H2'), 1.27–1.40 (4 H, m, H2, H4), 1.37 (9 H, s, Boc), 1.34 (9 H, s, Boc), 1.21 (2 H, m, H3).

*1-Amino-4,8-di(tert-butyloxycarbonyl)-13-(tert-butyloxycarbonylamino)-4,8-diazatridecane (**XII**).* A solution of (**XI**) (94.17 g, 0.184 mol) in anhydrous diethyl ether (150 ml) was added dropwise to a cooled to a –5°C suspension of $LiAlH_4$ (28.3 g, 0.744 mol) in anhydrous diethyl ether (1.8 l), stirred for 40 min at 0°C, treated dropwise with 2 M NaOH (93 ml), and additionally stirred for 4 h. The precipitate was filtered off and thoroughly washed on a filter with ether. Filtrates were combined and evaporated to give 80.7 g (85%) of (**XII**) in the form of a colorless oil; R_f 0.21 (D); ^{1}H NMR: 4.56 (1 H, bs, BocNH) 3.00–3.40 (10 H, m, H13, H9, H7, H5, H,3), 2.65 (2 H, m, H1), 1.72 (2 H, m, H6), 1.42–1.64 (6 H, m, H12, H10, H2), 1.42 (27 H, m, Boc), 1.26 (2 H, m, H11), 1.24 (2 H, s, NH_2).

N^{α}-Z-Arginine was obtained by the *L*-arginine treatment with BnOCOCl in aqueous $NaHCO_3$; yield 95%; mp 175°C [9]. Tris-Z-arginine was synthesized by silylation of N^{α}-Z-arginine with Me_3SiCl in the presence of $(Pr^i)_2EtN$ with the subsequent treatment with a small excess of BnOCOCl; yield 64%; mp 139°C; $[\alpha]_D^{25}$ +17.0 (*c* 1, $CHCl_3$) (lit. [10]: mp 138.9°C; $[\alpha]_D^{25}$ +17.1 (*c* 1, $CHCl_3$)). Tris-Z-arginine *p*-nitrophenyl ester (**XIII**) was obtained by the procedure in [11]; yield 84%; mp 135°C; $[\alpha]_D^{25}$ –8.1 (*c* 1, $CHCl_3$) (lit. [11]: yield 84%; mp 134–135°C; $[\alpha]_D^{25}$ –7.25 (*c* 1, $CHCl_3$)).

Boc-*L*-asparagine *p*-nitrophenyl ester ((**XIV**) was obtained from Boc-*L*-asparagine and *p*-nitrophenol in the presence of DCC in DMF by the procedure in [12]; yield 90%; mp 180°C; $[\alpha]_D^{25}$ –36 (*c* 1, DMF).

*15-(N-(2,4-Di(benzyloxy)phenylacetyl)-L-asparaginyl)-5,9-di(benzyloxycarbonyl)-1-(N,N,N-tris(benzyloxycarbonyl)-L-arginyl)-1,5,9,15-tetraazapentadecane (**XVII**).* Compound (**XIII**) (40.43 g, 58 mmol) was added to a stirred solution of (**XII**) (29.93 g, 58 mmol) in dry THF (280 ml). The reaction mixture was stirred for 24 h at room temperature and evaporated in a vacuum. The residue was dissolved in dichloromethane (300 m) and successively

washed with 5% $NaHCO_3$ (5 × 300 ml), 10% NaCl/3% ammonia (5 × 300 ml), and water (3 × 300 ml). The solution was dried with Na_2SO_4 and evaporated to give (**XV**); slightly yellow oil; yield 59.18 g (95%); R_f 0.65 (C); it was used without any additional purification.

A solution of (**XV**) (59.18 g, 55.1 mmol) in dry dichloromethane (280 ml) was treated under ice cooling with CF_3COOH (110 ml). The solution was stirred for 2 h at room temperature, evaporated in a vacuum, and the residue was dried in a vacuum for 4 h at 40°C. Dry THF (300 ml) was added and then, successively, triethylamine (38 ml) and (**XIV**) (19.77 g, 56 mmol) during cooling with ice. The reaction mixture was kept overnight at room temperature when monitoring the disappearance of (**XIV**) by TLC, R_f 0.38 (D); then twofold concentrated in a vacuum and treated with dry DMF (200 ml), Et_3N (30 ml); and, dropwise at cooling with ice, with BnOCOCl (29.0 g, 170 mmol). The reaction mixture was stirred overnight at room temperature, poured into 2 M ammonia (3 l), and stirred for 3 h. The precipitated solid was separated and washed on a filter with 2 M ammonia and water up to colorless washings. The precipitate was dissolved in dichloromethane (600 ml) and additionally washed with ammonia to the complete discoloration of the organic layer. The solvent was evaporated in a vacuum to give (**XVI**); yield 63.67 g (92%); R_f 0.45 (D).

Trifluoroacetic acid (45 ml) was added to a solution of (**XVI**) (49.29 g, 39.24 mmol) in dry chloroform (90 ml), and the mixture was stirred for 2 h at room temperature. The solution was evaporated in a vacuum, the residue was dried for 4 h at 40°C in a vacuum, anhydrous THF (160 ml) was added, and Et_3N (16 ml) and a solution of (**VI**) (17.27 g, 47.1 mmol) in THF (80 ml) were successively added under stirring and cooling with ice. The reaction mixture was left overnight, concentrated in a vacuum to a half of the volume, and poured into water (1500 ml). The precipitate was separated, thoroughly washed with water on a filter, dried in a vacuum over NaOH, and recrystallized from isopropanol to give (**XVII**) (55.34 g, 95%); R_f 0.51 (D); ^{1}H NMR: 7.20–7.45 (35 H, m, Ph), 6.94 (1 H, d, *J* 8.4, $H6_{Ar}$), 6.34 (1 H, d, *J* 2.4, $H3_{Ar}$), 6.25–6.30 (1 H, dd, *J* 2.4 and 8.4, $H5_{Ar}$), 4.90–5.15 (14 H, m, CH_2Ph), 3.98 (1 H, bs, $H1_{Arg}$), 3.80–3.88 (2 H, m, $ArCH_2CO$), 3.44 (2 H, d, *J* 4.8, $H5_{Arg}$), 3.10–3.19 (12 H, m, H2, H6, H8, H10, H14), 2.70–2.76 (1 H, dd, *J* 6.0 and 15.6, $H2_{Asn}$), 2.59–2.65 (2 H, dd, *J* 6.0 and 15.6, $H3_{Asn}$), 1.58–1.80 (10 H, m, H3, H7, H11 + $H3_{Arg}$, $H4_{Arg}$), 1.20–1.43 (4 H, m, H12, H13).

*15-(N-(2,4-Dihydroxyphenylacetyl)-L-asparaginyl)-1-L-arginyl-1,5,9,15-tetraazapenadecane (argiopine) (**I**).* A suspension of (**XVII**) (41.74 g, 28.09 mmol), 10% Pd/C (8.6 g), and trifluoroacetic acid (25 ml) in methanol (1100 ml) was stirred for 24 h in a hydrogen flow. The catalyst was separated, the filtrate was evaporated in a vacuum, and the residue was lyophilized from a water solution. Compound (**I**) (30.7 g) was obtained in the form of a salt with trifluoroacetic acid; the yield was practically quantitative; ^{1}H NMR: 6.96 (1 H, d, *J* 8.4, $H5_{Ar}$), 6.35 (1 H, d, *J* 2.4, $H3_{Ar}$), 6.30 (1 H, dd, *J* 2.4 and 8.4, $H6_{Ar}$), 4.62 (1 H, t, *J* 6.0, $H2_{Asn}$), 3.89 (1 H, t, *J* 6.5, $H2_{Arg}$), 3.45–2.80 (16 H, m, H2, H4, H6, H8, H10, H14, $H5_{Arg}$, CH_2Ar), 2.70 (2 H, d, *J* 6.0, $H3_{Asn}$), 2.07 (2 H, m, H7), 1.97–1.85 (4 H, m, H3, $H3_{Arg}$), 1.70–1.51 (6 H, m, H10, H13, $H4_{Arg}$), 1.48–1.29 (2 H, m, H12); MS, *m/z* 637 ($M + 1^+$).

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