177. Enantioselective Synthesis of (2*S*)-2-Amino-3-(4-hydroxy-3-phosphonophenyl)propionic Acid (= 3′-Phosphono-L-tyrosine) and Its Incorporation into Peptides

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The asymmetric synthesis of derivatives of the new amino acid (2*S*)-2-amino-3-(4-hydroxy-3-phosphonophenyl)propionic acid (3′-phosphono-L-tyrosine; Tyr [3′-PO(OH)$_2$]) is described. The protected amino acid **13** is obtained *via* a *Schöllkopf* synthesis by coupling of the rearranged *ortho*-phosphonophenolic side chain (see *Scheme 1*, **6a**) with the lithiated bis-lactim ether **8** of cyclo(-D-valyl-glycyl-) (see *Scheme 2*). The incorporation of the protected amino acid **14** in a biologically active dodecapeptide is successfully achieved by the [(9*H*-fluoren-9-yl)-methoxy]carbonyl (Fmoc) strategy of solid-phase peptide synthesis. Differential protection of Tyr[3′-PO(OH)$_2$] provides four levels of selective deprotection of, in the order, the N_2-amino, the carboxyl (cleavage from the resin), the phenol, and the phosphono function (→peptide **16**).

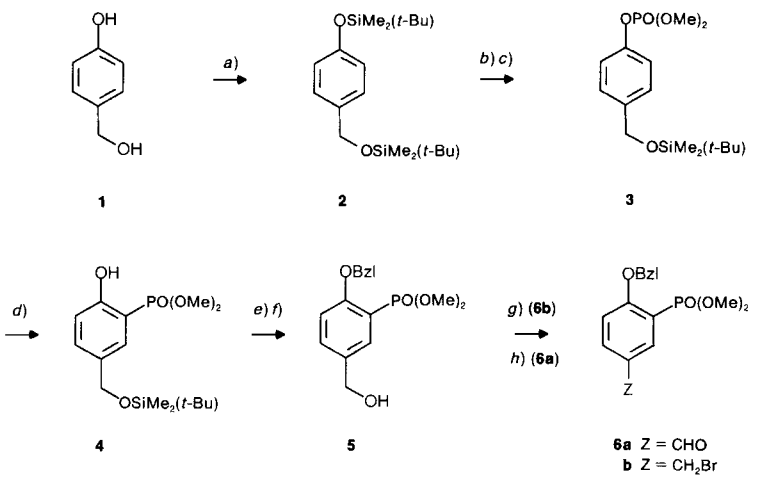
Introduction. – Phosphorylation of tyrosine residues in proteins plays an important role in signal-transduction pathways during cell transformation and hormone-induced cell growth [1] [2]. Together protein tyrosine kinases and phosphatases catalyze the reversible transfer of phosphate to tyrosyl residues of enzymes and other proteins. Peptides containing analogs of phosphotyrosine may serve either as product inhibitors of kinases or as substrate inhibitors of phosphatases and, therefore, have potential as anticancer drugs. Both as a building block during peptide synthesis and as a constitutive residue in the final peptide, phosphotyrosine suffers from being easily hydrolyzable under acidic conditions [3], and several attempts were made to stabilize the attachment of the phosphate group on the aryl moiety, including the replacement of the phenolic O-atom by a CH_2 or CF_2 group [4–6], or the replacement of the aryl C–O bond by a C–P bond [7]. However, these methods either introduce an additional methylene group between the aromatic ring and the phosphate group or eliminate the phenolic function of tyrosine. We report here on the enantioselective preparation of a new phosphono derivative of tyrosine in which the free phenolic function is maintained in position 4′ and the phosphono group attached to the aromatic ring in position 3′ by a stable C–P bond. We describe several protected derivatives suitable for peptide synthesis and the incorporation of one derivative into a C-terminal fragment of the anticoagulant protein hirudin [8].

Results. – 1. *Synthesis.* The key step in the synthesis was a base-promoted rearrangement of an aryl phosphate to a 2-hydroxy-3-arylphosphonate. This known rearrangement [9] [10] of phenol-derived phosphates failed when directly attempted on protected tyrosine, *e.g.*, on Boc-Tyr[O^4-PO(OEt)$_2$]-OBu^t. For this reason, another strategy was devised in which the 2-hydroxyarylphosphonate moiety was prepared separately, using the given rearrangement (*Scheme 1*), and then included as the incoming side chain in an

asymmetric amino-acid synthesis starting with the bis-lactim ether of cyclo(-D-valyl-glycyl-) as the chiral agent [11] (*Scheme 2*).

The side-chain synthon **6a** or **6b** was prepared from 4-(hydroxymethyl)phenol (**1**) *via* the fully protected (*tert*-butyl)dimethylsilyl derivative **2** which was selectively deprotected (F^- ions) and phosphorylated at the phenolic OH group with dimethyl phosphite [12] to give **3** (*Scheme 1*). The rearrangement **3** → **4** was then successfully carried out under the conditions which did not work for protected tyrosine. Subsequent benzylation of the phenolic OH group and desilylation afforded **5** which was either brominated to **6b** in poor yields (18% with CBr_4/PPh_3 or 38% with allyl bromide and 1,1′-carbonylbis(1*H*-imidazole)) or oxidized with activated MnO_2 [13] to aldehyde **6a** in 96% yield (38% overall from **1**).

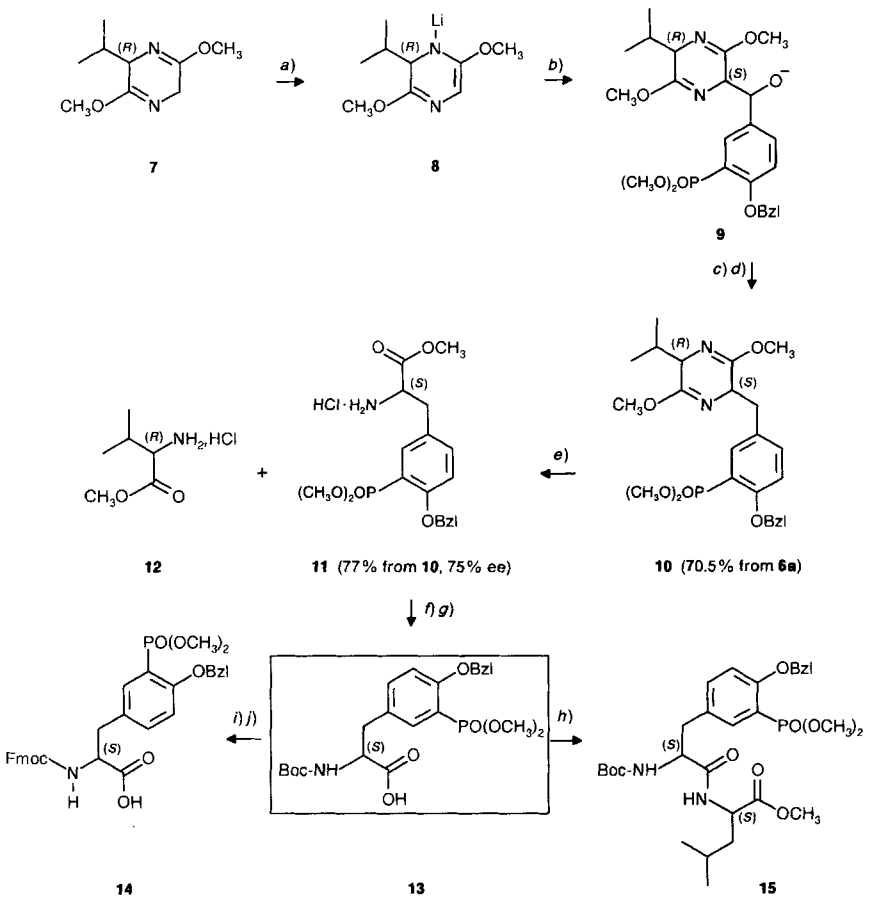
Scheme 1



a) (*t*-Bu)Me_2SiCl, 1*H*-imidazole, DMF, 20°, 48 h. *b*) 1 Equiv. of Bu_4NF, THF, 0°. *c*) $HOP(OMe)_2$, dicyclohexylamine, CCl_4, 70°. *d*) LDA, THF, −78°. *e*) 1. NaH, THF, 0°; 2. $PhCH_2Br$, DMF, cat. $MgBr_2$. *f*) 2 Equiv. of Bu_4NF, THF, 20°. *g*) Allyl bromide, 1,1′-carbonylbis(1*H*-imidazole), MeCN, 80°. *h*) MnO_2, THF, 20°.

The amino-acid backbone was obtained from the bis-lactim ether **7** (*Scheme 2*) of cyclo(-D-Val-Gly-) prepared according to *Schöllkopf* [11], except for the use of trichloromethyl chloroformate [14] instead of gaseous phosgene in the first step. The enantiomeric purity of **7** was higher than 99% as checked by HPLC on a chiral phase using the corresponding optically pure L-enantiomer as reference (*Merck*). The bis-lactim ether was first regiospecifically metalated on glycine by treatment with BuLi and then tentatively condensed to bromide **6b**, without success. In contrast, aldehyde **6a** reacted readily with lithium salt **8** to give the intermediate **9** which was converted to **10** by direct trapping with *O*-phenyl chlorothiocarbonate [15] followed by reduction with Bu_3 SnH (71% overall yield from **8**). Mild acid hydrolysis liberated then, as expected, the L-amino acid methyl ester **11** (77% yield; 55% from **7**), together with methyl D-valinate (**12**) from which it was separated by flash chromatography (FC). The enantiomer excess of **11** was estimated by preparing its N_2-Boc derivative **13** and coupling it with L-leucine methyl

Scheme 2



a) BuLi, THF, −78°. *b*) **6a**, THF, −78°. *c*) PhOCSCl, 20°. *d*) Bu_3SnH, AIBN, toluene, 70°. *e*) 2 Equiv. of 0.25N HCl, 48 h. *f*) Boc_2O, Et_3N, $CHCl_3$, 20°. *g*) 1 Equiv. of NaOH, MeOH, 12 h. *h*) HCl·Leu-OMe, DCC, BtOH, Et_3N, DMF. *i*) 10% CF_3COOH/CH_2Cl_2, 0–20°. *j*) FmocCl, dioxane, Na_2CO_3.

ester under usual non-racemizing conditions. The dipeptide **15** was obtained in 77% yield, and HPLC analysis of the diastereoisomers showed a diastereoisomer excess of 75% (*Fig. 1*). Mild acid treatment of **13** in CF_3COOH (Boc deprotection) followed by reaction with [(9*H*-fluoren-9-yl)methoxy]carbonyl chloride (FmocCl) gave the protected 3′-phosphono-L-tyrosine **14** suitable for the Fmoc strategy of peptide synthesis [16].

To confirm the compatibility of the protecting groups on the phosphono substituent of tyrosine with the protocol of peptide synthesis on solid phase [17], compound **14** was introduced into an analog of the C-terminal fragment of hirudin. The corresponding position is occupied by tyrosine O^4-sulfate in natural hirudin [8]. The usual conditions used for cleavage of the peptide from the resin left intact the protecting groups on the phosphono and OH group of tyrosine. Catalytic hydrogenation removed the benzyl

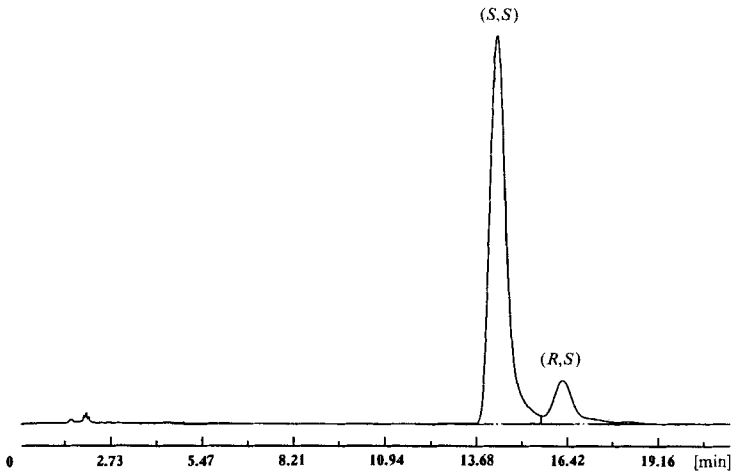


Fig. 1. *HPLC Analysis of the protected dipeptide derivative* **15**. Conditions: 10 µg applied; *Lichrosorb SI60* (5 µm, 4.6 × 150 mm); EtOH/heptane (1:9), flow rate 1 ml/min; detector 205 nm. (*S,S*)-Diastereoisomer: t_R 14.29 min, rel. area 87.4%. (*R,S*)-Diastereoisomer: t_R 16.26 min, rel. area 12.6%.

group, and treatment with CF_3COOH in the presence of Me_3SiBr hydrolyzed the methyl ester functions on tyrosine. The final phosphono-peptide (guanidinobenzoyl)-Gly-Asp-Phe-Glu-Abo-Ile-Pro-Glu-Glu-Tyr[3′-PO(OH)$_2$]-Leu-D-Glu-OH (**16**), in which Abo was the residue of (*S*)-2-azabicyclo[2.2.2]octane-3-carboxylic acid [18], was obtained as a pure lyophilisate, as confirmed by reversed-phase HPLC (*Fig. 2*), amino-acid analysis, and FAB-MS.

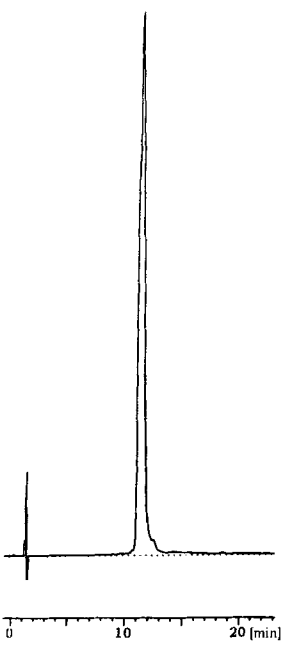


Fig. 2. *HPLC Analysis of peptide* **16**. Conditions: *Milipore Delta Pak* (5 µm, C_{18} 300 Å, 3.9 × 150 mm); 20→40% MeCN/H_2O (0.01% CF_3COOH) gradient within 30 min, flow rate 1 ml/min; detection 210 nm.

2. *Biological Activity.* The hirudin analog displayed as high an anticoagulant potency *in vitro* (*CTT 2* = 3.2 ± 0.4 μM, concentration of peptide that doubles thrombin time) and duration of action *in vivo* (increase of prothrombin time by a factor *Fm* = 2.03 ± 0.15 after 15 min at the dose of 8 mg/kg, rat, i.v.) as one of the most potent reference compounds, succinyl-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-D-Glu-OH (MDL 28050, Cha = cyclohexyl-L-alanine) [8], for which the values under the same conditions were *CTT2* = 2.4 ± 0.4 μM and *Fm* = 2.14 ± 0.15.

Discussion. – The enantiomeric excess obtained in the condensation of the side-chain synthons **6a** or **6b** with the metalated partner **8** is of major importance. Alternative to our route *via* the nonreactive bromide **6b** could be the tosylate, mesylate, or triflate of **5**, for which good asymmetrical induction was described in analogous syntheses [19] [20]. Similarly, with an aldehyde, an improved enantiomer excess was obtained when Ti instead of Li was the metal in **8** [21] using the not commercially available reagent chlorotris[dimethylamino]titanium for metallation.

The amino acid (2*S*)-2-amino-3-(4-hydroxy-3-phosphonophenyl)propionic acid (3′-phosphono-L-tyrosine) is a new residue which can be obtained *via* an asymmetric synthesis in good yield as derivatives with removable protecting groups and conveniently introduced into biologically active peptides. Four levels of selective deprotection are possible, in the following order: with 25% piperidine in DMF for the N^2-Fmoc group, with 75% CF_3COOH/CH_2Cl_2 for *t*-Bu-type groups and cleavage from the resin, with catalytic hydrogenation for the benzyl ether at the phenolic group, and with 12% Me_3SiBr/CF_3COOH for the phosphonic acid methyl ester. The duration of action *in vivo* of the phosphono-peptide **16** as long as that of MDL 28050, a peptide of comparable length which does not contain a phosphorylated side chain, confirms the often reported property of phosphonic acids to be non-hydrolyzable phosphate mimics.

Thanks are due to Dr. *Ph. Hennig* and to Dr. *J. P. Volland* and collaborators for expert analytical characterization of the products.

Experimental Part

General. The starting material 4-(hydroxymethyl)phenol (**1**) and (2*S*)-2,5-dihydro-2-isopropyl-3,6-dimethoxypyrazine (*ent*-**7**) were purchased from *Merck*. The reagents were from *Fluka AG*, Fmoc-amino acids from *Bachem AG*. Peptide synthesis was performed on a *Milligen-9050* continuous-flow apparatus with photometric monitoring. Peptide **16** was purified by HPLC on *Waters-Prep-LC-3000* system on a *PrePak*® cartridge (47 × 300 mm) filled with a C_{18}-silica (300 Å, 15 μ) phase, and its molecular weight determined by FAB-MS on a *Nermag-R10-10C* apparatus (glycerol/thioglycerol matrix; ionization with Kr atoms). For amino-acid analysis, the peptide was hydrolyzed in 6N HCl for 20 h at 110° and the hydrolyzate automatically analyzed in a *Varian-LC-90-Star* system, after transformation to the Fmoc derivatives and separation on an *Aminotag-C18* (5 μm) phase. Specific rotations (*C* in g/100 ml): *Perkin-Elmer-241* polarimeter. ^{1}H-NMR Spectra: *Bruker* spectrometers at 200 or 300 MHz with tetramethylsilane as external standard.

*4-{[(*tert*-Butyl)dimethylsilyloxy]methyl}phenyl Dimethyl Phosphate* (**3**). The soln. of **1** (5 g, 40.3 mmol) in DMF (50 ml), (*t*-Bu)Me_2SiCl (18.2 g, 120 mmol), and 1*H*-imidazole (16.5 g, 240 mmol) were stirred for 48 h at r.t. After evaporation, the resulting two phases were taken up into Et_2O, and the white insoluble residue was discarded. The concentrated filtrate was then submitted to FC (silica gel, AcOEt/pentane 1:39): 12.4 g (87%) of **2**. Colourless oil. R_f (AcOEt/pentane 1:39) 0.5. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.09 (*s*, 6 H); 0.19 (*s*, 6 H); 0.94 (*s*, 9 H); 0.99 (*s*, 9 H); 4.68 (*s*, 2 H); 6.8 (*dd*, 2 H); 7.2 (*dd*, 2 H).

To the soln. of **2** (12 g, 29.4 mmol) in THF (200 ml), 1M Bu_4NF in THF (29.4 ml) was slowly added under cooling at 0°. After 20 min (TLC: no **2** left), sat. aq. NH_4Cl soln. (250 ml) was added, the product extracted with

Et_2O, the extract dried (Na_2SO_4) and evaporated and the oil mixed with dicyclohexylamine (6.4 ml, 32 mmol) and refluxed for 1 h in acetone (20 ml). This solvent was then replaced with CCl_4 (150 ml) and dimethyl phosphite (3 ml, 32 mmol) added under stirring. The mixture was refluxed for 4 h, then cooled and filtered and the solvent evaporated. The residual oil afforded, after FC (silica gel, AcOEt/pentane 1:1), 8.3 g (70%) of pure **3**. R_f (AcOEt/pentane 1:1) 0.52. IR (film): 995 (P–O), 1065 (Si–O–C), 1290 (P=O). ^{1}H-NMR (200 MHz, $CDCl_3$): 0.01 (*s*, 6 H); 0.83 (*s*, 9 H); 3.75 (*d*, 6 H); 4.6 (*s*, 2 H); 7.06 (*dd*, 2 H); 7.19 (*dd*, 2 H). Anal. calc. for $C_{15}H_{27}O_5PSi$ (346.4): C 52.01, H 7.86; found: C 51.61, H 7.62.

*Dimethyl 2-Hydroxy-5-{[(*tert*-butyl)dimethylsilyloxy]methyl}phenylphosphonate* (**4**). At −78°, 2.5M BuLi in hexane (11.5 ml, 34.2 mmol) was added to $(i\text{-}Pr)_2NH$ (4 ml, 28.5 mmol) in THF (50 ml) under N_2. After stirring for 15 min, **3** (8.2 g, 23 mmol) dissolved in THF (100 ml) was slowly added and stirring continued for 1 h at −78° and 1 h at r.t. The mixture was then poured into sat. aq. NH_4Cl soln. (400 ml) and extracted 3 times with Et_2O. Drying (Na_2SO_4) and evaporation gave an oil which was purified by FC (silica gel, AcOEt/pentane 1:1): 6.4 g (77%) of **4**. Oil. R_f (AcOEt/pentane 1:1) 0.60. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.0 (*s*, 6 H); 0.85 (*s*, 9 H); 3.68 (*d*, 6 H); 4.55 (*s*, 2 H); 6.89 (*m*, 1 H); 7.22 (*m*, 1 H); 7.35 (*m*, 1 H). Anal. calc. for $C_{15}H_{27}O_5PSi$ (346.4): C 52.01, H 7.86; found: C 52.26, H 7.61.

Dimethyl 2-(Benzyloxy)-5-(hydroxymethyl)phenylphosphonate (**5**). Small portions of NaH (0.8 g, 20 mmol) were added within 30 min to a soln. of **4** (6.3 g, 18.2 mmol) in THF (250 ml) at 0°. After removal of THF and dissolution of the residue in DMF (100 ml), benzyl bromide (2.4 ml, 20 mmol) was introduced together with a catalytic amount of $MgBr_2$. Stirring was continued for 15 h at r.t., DMF removed, and the residue taken up into H_2O and Et_2O. The combined Et_2O extracts gave, after drying (Na_2SO_4) and evaporation, an oil which was purified by FC (silica gel, AcOEt/pentane 1:1): 6.8 g (85%) of (*tert*-butyl)dimethylsilylated 2-(benzyloxy)phenylphosphonate. This intermediate was dissolved in THF (80 ml) and treated with 1M Bu_4F in THF (31 ml) for 25 min (→gel). FC (silica gel, AcOEt then MeOH) gave 5 g (85%) of **5**. R_f ($CHCl_3$/MeOH/H_2O/AcOH 89:9:1:0.5) 0.54. IR (film): 1252 (P=O), 3373 (OH). ^{1}H-NMR (200 MHz, $CDCl_3$): 3.72 (*d*, 6 H); 4.60 (*s*, 2 H); 5.17 (*s*, 2 H); 6.95 (*dd*, 1 H); 7.35 (*m*, 3 H); 7.50 (*m*, 3 H); 7.85 (*dd*, 1 H). Anal. calc. for $C_{16}H_{19}O_5P$ (322.3): C 59.63, H 5.94; found: C 59.47, H 6.03.

Dimethyl 2-(Benzyloxy)-5-formylphenylphosphonate (**6a**). A soln. of **5** (4 g, 12.4 mmol) in THF (100 ml) and MnO_2 (10.8 g, 124 mmol) were stirred for 1.5 h at r.t. The mixture was filtered through *Celite* and the solid washed with THF. Evaporation of the solvent afforded pure **6a** (3.8 g, 96%). R_f (acetone/CH_2Cl_2 1:1) 0.74. IR (film): 1261 (P=O), 1689 (C=O). ^{1}H-NMR (200 MHz, $CDCl_3$): 3.80 (*d*, 6 H); 5.29 (*s*, 2 H); 7.10 (*dd*, 1 H); 7.40 (*m*, 3 H); 7.50 (*m*, 2 H); 8.0 (*dd*, 1 H); 8.3 (*dd*, 1 H); 9.91 (*s*, 1 H). Anal. calc. for $C_{16}H_{17}O_5P$ (320.3): C 60.00, H 5.35; found: C 59.58, H 5.46.

Dimethyl 2-(Benzyloxy)-5-(bromomethyl)phenylphosphonate (**6b**). Allyl bromide (3.7 ml, 43.5 mmol) was added to the soln. of **5** (0.93 g, 2.9 mmol) and of 1,1′-carbonylbis(1*H*-imidazole) (0.49 g, 3 mmol) in dry MeCN (5 ml). The mixture was stirred for 30 min at r.t. and then refluxed for 1.5 h. Evaporation followed by FC (silica gel, AcOEt) yielded 0.42 g (38%) of **6b**. White solid. R_f (AcOEt) 0.47. IR (nujol): 1159 (P–O), 1232 (P=O). ^{1}H-NMR (200 MHz, $CDCl_3$): 3.73 (*d*, 6 H); 4.44 (*s*, 2 H); 5.17 (*s*, 2 H); 6.95 (*dd*, 1 H); 7.40 (*m*, 1 H); 7.50 (*m*, 5 H); 7.85 (*dd*, 1 H). Anal. calc. for $C_{16}H_{18}BrO_4P$ (385.2): C 49.89, H 4.71, Br 20.74; found: C 50.08, H 4.66, Br 21.00.

Dimethyl 2-(Benzyloxy)-5-{[(2S,5R)-2,5-dihydro-5-isopropyl-3,6-dimethoxypyrazin-2-yl]methyl}phenylphosphonate (**10**). BuLi (1.6M in hexane; 3.6 ml, 5.8 mmol) was added to a soln. of (2*R*)-2,5-dihydro-2-isopropyl-3,6-dimethoxypyrazine (**7**; 1 g, 5.3 mmol) in THF (10 ml) at −78°. After stirring for 30 min, **6a** (1.7 g, 5.3 mmol) in THF (10 ml) was added dropwise and stirring continued for 5 h at −78° and 14 h at r.t. The mixture was then directly treated with *O*-phenyl chlorothiocarbonate (0.8 ml, 5.8 mmol) and kept under agitation for 1 h at r.t. Addition of aq. NH_4Cl soln. (100 ml) and extraction into Et_2O followed by washing of the org. phase with aq. $NaHCO_3$ soln., drying (Na_2SO_4), and evaporation yielded an oily residue which was taken up in toluene (120 ml) and degassed with a N_2 stream for 20 min. The soln. was then treated with Bu_3SnH (2 ml, 7.4 mmol) in presence of 2,2′-azobis(isobutyronitrile) (= 2,2′-dimethyl-2,2′-azobis(propanenitrile); 0.16 g, 1 mmol), heated to 75° for 1.5 h, and then stirred at r.t. overnight. The residue obtained after evaporation was subjected to FC (silica gel, AcOEt/pentane 9:1): 1.7 g (70.5%) of **10**. Oil. R_f (AcOEt/pentane 9:1) 0.57. $[\alpha]_D^{22} = +11.2$ (*c* = 1, MeOH). IR (film): 1250 (P=O), 1695 (C=N), 1603 (C=C). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.59 (*d*, 3 H); 0.91 (*d*, 3 H); 2.12 (*m*, 1 H); 3.0 (*m*, 2 H); 3.20 (*dd*, 1 H); 3.60–3.65 (*s*, *d*, 12 H); 4.30 (*m*, 1 H); 5.10 (*s*, 2 H); 6.85 (*m*, 1 H); 7.20 (*dd*, 1 H); 7.30 (*m*, 4 H); 7.50 (*m*, 1 H); 7.60 (*dd*, 1 H). Anal. calc. for $C_{25}H_{33}N_2O_6P$ (488.5): C 61.47, H 6.80, N 5.73; found: C 61.05, H 6.57, N 5.55.

Methyl (2S)-2-Amino-3-[4-(benzyloxy)-3-(dimethoxyphosphoryl)phenyl]propionate Hydrochloride (**11**). A suspension of **10** (1.2 g, 2.45 mmol) in 0.25N HCl (20 ml, 4.9 mmol) was vigorously stirred for 100 h at r.t. After evaporation, **11**/**12** were separated by FC ($CHCl_3$/MeOH/H_2O/AcOH 88.5:9:1:0.5): 0.81 g (77%) of **11** which eluted first. R_f (same system) 0.57. $[\alpha]_D^{22} = +2.4$ (*c* = 0.7, MeOH). IR (film): 1032 and 1254 (P=O), 1604 (C=C),

1739 (C=O, ester), 3379 (NH_3^+). ^{1}H-NMR (300 MHz, $CDCl_3$): 2.90 (*dd*, 1 H); 3.10 (*dd*, 1 H); 3.30 (*s*, 2 H); 3.70 (*m*, 10 H); 5.16 (*s*, 2 H); 6.92 (*dd*, 1 H); 7.30 (*m*, 4 H); 7.50 (*m*, 2 H); 7.70 (*dd*, 1 H). FAB-MS: 394 (MH^+). Anal. calc. for $C_{19}H_{22}NO_6P \cdot HCl$ (427.8): C 53.34, H 5.42, N 3.27, Cl 8.29; found: C 53.10, H 5.79, N 3.33, Cl 8.79.

4′-O-Benzyl-N^2*-[(*tert*-butyloxy)carbonyl]-3-(dimethoxyphosphoryl)*-L-*tyrosine* (**13**). Et_3N (0.41 ml, 3 mmol) and $(Boc)_2O$ (0.36 g, 1.64 mmol) were added to a soln. of **11** (0.64 g, 1.5 mmol) in $CHCl_3$ (30 ml) at 0°. The mixture was kept for 1 h under stirring at 0° and for 14 h at r.t., then washed with H_2O, 5% aq. citric-acid soln., 5% aq. $NaHCO_3$ soln., and H_2O, the org. phase dried and evaporated, and the intermediate methyl carboxylate isolated by FC (silica gel, toluene/EtOH 95:5). The solid residue (0.48 g) was taken up in MeOH (15 ml), treated with 1N NaOH (1 ml) at 0°, and stirred for 2 h at 0°. After evaporation at < 25°, the residue was partitioned in CH_2Cl_2/H_2O and the product extracted into the org. phase at pH 2. The extract was purified by FC (silica gel, $CHCl_3$/MeOH 8:2): 0.33 g (81%) of pure **13**. White solid. M.p. 65°. R_f ($CHCl_3$/MeOH 8:2) 0.5. $[\alpha]_D^{22} = +22.7$ ($c = 1$, MeOH). ^{1}H-NMR (200 MHz, (D_6)DMSO): 1.30 (*s*, 9 H); 2.70 (*dd*, 1 H); 3.10 (*dd*, 1 H); 3.60 (*d*, 6 H); 3.85 (*m*, 1 H); 5.10 (*s*, 2 H); 6.20 (*dd*, 1 H); 7.0 (*dd*, 1 H); 7.35 (*m*, 6 H); 7.50 (*dd*, 1 H). Anal. calc. for $C_{23}H_{30}NO_8P$ (479.5): C 57.62 H 6.31, N 2.92; found: C 57.34, H 5.92, N 3.04.

4′-O-Benzyl-N^2*-{[(9H-fluoren-9-yl)methoxy]carbonyl}-3-(dimethoxyphosphoryl)*-L-*tyrosine* (**14**). Treatment of **13** (0.12 g, 0.25 mmol) in CH_2Cl_2 (4.5 ml) with CF_3COOH (0.5 ml) at 0° was followed by stirring for 2.5 h at 0° and 1 h at r.t. Toluene (20 ml) was then added, the mixture evaporated, and the residue taken up in dioxane (1.5 ml). The soln. cooled to 0° was treated with 10% aq. Na_2CO_3 soln. (2 ml) and FmocCl (0.07 g, 0.27 mmol) and the reaction allowed to proceed for 2 h at 0° and 1 h at r.t. After the addition of H_2O (50 ml), the mixture was extracted with Et_2O (3 times) and the org. phase discarded. The H_2O phase was brought to pH 2 ($KHSO_4/K_2SO_4$) and the product extracted into Et_2O (3 times). The oily residue obtained after drying and evaporation was triturated in pentane: 0.14 g (92%) of **14**. White solid. M.p. 85°. R_f ($CHCl_3$/MeOH/H_2O/AcOH 70:25:4.5:0.5) 0.67. $[\alpha]_D^{22} = -1.7$ ($c = 0.17$, MeOH). ^{1}H-NMR (200 MHz, $CDCl_3$): 3.70 (*m*, 6 H); 3.2–4.15 (*m*, 2 H); 4.30 (*m*, 1 H); 4.80 (*m*, 1 H); 5.15 (*s*, 2 H); 6.10 (*d*, 1 H); 6.85 (*t*, 1 H); 7.2–7.8 (*m*, 15 H). Anal. calc. for $C_{33}H_{32}NO_8P$ (601.5): C 65.89, H 5.35, N 2.33; found: C 65.42, H 4.93, N 2.52.

Boc-Tyr[$O^{4'}$*-Bzl,3′-PO(*OMe_2*)]-Leu-OMe* (**15**). The soln. in CH_2Cl_2 (15 ml) of **13** (0.2 g, 0.42 mmol), H-Leu-OMe·HCl (91 mg, 0.5 mmol), Et_3N (0.07 ml, 0.5 mmol), and 1*H*-benzotriazol-1-ol (BtOH, 77 mg, 0.5 mmol) was stirred for 30 min at r.t. *N,N′*-Dicyclohexylcarbodiimide (DCC; 113 mg, 0.55 mmol) was then added and the mixture allowed to react for 3.5 h at r.t. (TLC: no **13** left). After filtration, the org. phase was washed with H_2O, 5% aq. citric-acid soln., and 5% aq. $NaHCO_3$ soln., dried (Na_2SO_4), and evaporated. A further separation of residual dicyclohexylurea was achieved on cooling an AcOEt soln. of the residue, after which the filtrate was submitted to FC (silica gel, AcOEt/pentane 8:2): 194 mg (77%) of **15** (diastereoisomer mixture). According to HPLC, the ratio of the diastereoisomers (*S,S*)/(*R,S*) was 87.4:12.6 and the diastereoisomer excess 75%. HPLC: t_R 14.29 and 16.26 min, resp. (for conditions, see *Fig. 1*). $[\alpha]_D^{22} = -6.2$ ($c = 0.34$, MeOH). ^{1}H-NMR (300 MHz, (D_6)DMSO): 0.85 (*d*, 3 H); 0.95 (*d*, 3 H); 1.30 (*s*, 9 H); 1.60 (*m*, 3 H); 2.70 (*dd*, 1 H); 2.90 (*dd*, 1 H); 3.60 (*d*, 6 H); 3.65 (*s*, 3 H); 4.15 (*m*, 1 H); 4.35 (*m*, 1 H); 5.20 (*s*, 2 H); 6.90 (*d*, 1 H); 7.15 (*dd*, 1 H); 7.30 (*m*, 1 H); 7.40 (*m*, 2 H); 7.50 (*m*, 3 H); 7.55 (*dd*, 1 H); 8.30 (*d*, 1 H). Anal. calc. for $C_{30}H_{43}N_2O_9P$ (606.7): C 59.40, H 7.14, N 4.62; found: C 59.33, H 7.28, N 4.57.

(Guanidinobenzoyl)-Gly-Asp-Phe-Glu-Abo-Ile-Pro-Glu-Glu-Tyr[3′-PO(OH)$_2$*]-Leu-*D-*Glu-OH Trifluoroacetate* (**16**; CF_3COOH; Abo = residue of (3*S*)-2-Azabicyclo[2.2.2]octane-3-carboxylic acid). The protected peptide (guanidinobenzoyl)-Gly-Asp(OBu^t)-Phe-Glu(OBu^t)-Abo-Ile-Pro-Glu(OBu^t)-Glu(OBu^t)-phosphoryl-Tyr[$O^{4'}$-Bzl, 3′-PO(OMe_2)]-Leu-D-Glu(OBu^t)-resin was assembled starting with Fmoc-D-Glu(OBu^t)-*p*-alkoxybenzylalcohol-resin (0.45 g, 0.18 mmol) and using 20% piperidine in *N,N′*-dimethylformamide (DMF, 2 × 15 min) for deprotection and BtOH/DCC or BtOH (i-Pr)$_2$EtN/TBTU (TBTU = 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate) for coupling (90 min). The protected amino acids (3 equiv.) were introduced in the following order: Fmoc-Leu-OH, Fmoc-Tyr[$O^{4'}$-Bzl,3′-PO(OMe_2)]-OH (**14**), Fmoc-Glu(OBu^t)-OH, Fmoc-Glu(OBu^t)-OH, Fmoc-Pro-OH, Fmoc-Ile-OH, Fmoc-Abo-OH, Fmoc-Glu(OBu^t)-OH, Fmoc-Phe-OH, Fmoc-Asp(OBu^t)-OH, and *N*-(guanidinobenzoyl)glycine. Cleavage from the resin and removal of the OBu^t groups was achieved in CF_3COOH/CH_2Cl_2/anisole/ethanedithiol 24:6:1:1 (32 ml) for 90 min at r.t. The solid residue obtained from the filtrate after evaporation was subjected to catalytic hydrogenation over 10% Pd/C in MeOH/H_2O 3:1 for 24 h at r.t. After filtration over *Celite* and evaporation, the residue was treated for 5 h at 0° with CF_3COOH (6 ml), Me_3SiBr (0.8 ml), and thioanisole (0.7 ml). The fully deprotected peptide was then submitted to prep. reversed-phase HPLC (gradient 20–40% *B* in *A* in 30 min, *A* = 0.1% CF_3COOH/H_2O, *B* = 0.1% CF_3COOH/MeCN): t_R 11.6 min. Yield 42 mg (12%). $[\alpha]_D^{22} = -18.78$ ($c = 0.2$, AcOH). Amino-acid analysis: Asp 0.97 (1), Glu 3.78 (4), Gly 1.10 (1), Pro 1.03 (1), Phe 1.05 (1), Ile + Abo 1.94 (2), Leu 1.00 (1). FAB-MS: 1718 (MH^+, calc. for *M* ($C_{77}H_{104}N_{15}O_{28}P$) 1717.7).

Pharmacological Methods. The anticoagulant activity was assessed *in vitro* and *ex vivo* according to *Broersma et al.* [22] and expressed as the concentration of the anticoagulant peptide required for doubling the thrombin time (*CTT2*) *in vitro* (human blood plasma) and as the factor by which the prothrombin time was increased *ex vivo* when the dose of 8 mg/kg is administered *in vivo* to the rat by the intravenous (i.v.) route. The experiments were carried out by *S. Simonet* and *T. J. Verbeuren* in our Institute.

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