washing of the resin with H_2O (2 × 10 mL). The filtrate was concentrated to give 100 mg (100%) of an oil that crystallized under vacuum. ^1H NMR indicated ~97% stereochemical purity of (-)-**2**:[38] mp 183-184.5 °C; $[\alpha]^{21}_D$ -170° (1.18, H_2O). There was no depression of melting point with authentic (-)-**2**:[39] mp 185-186 °C [lit.[40] mp 183-184 °C (corrected)]; $[\alpha]^{21}_D$ -184° (1.17, H_2O) [lit.[41] $[\alpha]^{18}_D$ -184° (4.03, H_2O)].

Acknowledgment. We are grateful to the National Institutes of Health, Grant GM 31958, for financial support. We thank Professor George M. Whitesides for helpful suggestions.

Registry No. (-)-**1**, 617-12-9; (-)-**2**, 138-59-0; (+)-**3**, 106861-59-0; (-)-**3**, 106861-60-3; (±)-**3**, 76947-23-4; (+)-**4**, 106861-61-4; (-)-**4**, 76985-84-7; (±)-**4**, 76985-85-8; (±)-**5a**, 106780-56-7; (±)-**5b**, 106780-57-8; (±)-**5c**, 106780-58-9; (±)-**5d**, 106780-59-0; (±)-**5e**, 106780-60-3; (±)-**5f**, 106780-61-4; (±)-**5g**, 106780-62-5; (±)-**5h**, 106780-63-6; (±)-**6a**, 106861-62-5; (-)-**6a**, 106861-65-8; (±)-**6b**, 106861-63-6; (-)-**6b**, 106861-66-9; (±)-**6c**, 106861-64-7; **7**, 106780-64-7; (-)-**8**, 106780-65-8; (±)-**8**, 106861-67-0; **9**, 106861-68-1; (±)-**10**, 106780-66-9; **11**, 106780-67-0; (-)-**12**, 106861-69-2; (-)-**13**, 106861-70-5; (-)-**14**, 106861-71-6; (-)-**15**, 40983-58-2; E.C.3.1.1.3, 9001-62-1; E.C.3.1.1.13, 9026-00-0.

Supplementary Material Available: ^1H NMR spectral data for **5a,c-i** and **6c-d** (2 pages). Ordering information is given on any current masthead page.

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Synthesis of Oligophosphopeptides and Related ATP γ-Peptide Esters as Probes for cAMP-Dependent Protein Kinase

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Received September 25, 1986

The synthesis of N- and C-terminally blocked heptapeptide substrates for cAMP-dependent protein kinase and the corresponding phosphoheptapeptide products has been accomplished by solution-phase techniques. The chemically synthesized heptapeptides were evaluated as substrates for the enzyme. ATP γ-peptide esters incorporating portions of the peptide substrate sequence also have been synthesized. The synthesis of Ac-Leu-Arg(NO_2)-Arg(NO_2)-Ala-(*O*-phospho)Ser-Leu-Gly-OMe, a potential precursor to the corresponding Arg-containing ATP γ-peptide ester, has been investigated by attempted displacement of peptide mesylates with tetra-*n*-butylammonium di-*tert*-butyl phosphate and by 85% phosphoric acid ring-opening of aziridine peptides.

Introduction

The heptapeptide Leu-Arg-Arg-Ala-Ser-Leu-Gly, also known as the "kemptide", is an excellent substrate for cAMP-dependent protein kinase,[1] being phosphorylated with kinetic parameters comparable to those of natural protein substrates.[2] It has thus received much attention in the last decade and has greatly facilitated the study of this enzyme. The corresponding phosphopeptide, Leu-Arg-Arg-Ala-(*O*-phospho)Ser-Leu-Gly, is important for product identification in the biochemical phosphorylation of the kemptide as well as for product inhibition studies of protein kinase and has thus far been obtained only through enzymatic means.[3,4] The enzymatic syntheses, however, are limited in scale and require additional purification steps. Solid-phase techniques of peptide synthesis, which have been successfully employed in the preparation of Leu-Arg-Arg-Ala-Ser-Leu-Gly,[2] have not been widely used in the synthesis of phosphopeptides.[5] Furthermore, certain commercial solid-phase preparations of the kemptide have been shown to contain significant impurities.[6] We report here the synthesis of the related peptides **1a** and **1b**, in which both the N- and C-termini are blocked, and the corresponding phosphopeptides **2a** and **2b**. The solution-phase methods employed in this work allow for the complete characterization of every intermediate in the synthetic scheme and make large-scale preparations feasible. The availability of blocked peptide kinase substrates and the putative phosphopeptide products should facilitate a variety of kinetic and spectroscopic studies of the protein kinase-catalyzed reaction and allow for a more extensive evaluation of blocked vs. free peptides as kinase substrates.[7,8] We also have investigated the synthesis of the phosphopeptide Ac-Leu-Arg(NO_2)-Arg(NO_2)-Ala-(*O*-phospho)Ser-Leu-Gly-OMe (**3**) as a potential intermediate in the synthesis of ATP γ-peptide esters, which have been proposed as multisubstrate adduct inhibitors of this enzyme.[9]

Ac-Leu-Arg-Arg-Ala-Ser-Leu-Gly-R

1a, R = OMe
b, R = NHMe

Ac-Leu-Arg-Arg-Ala-Ser(OPO$_3$H$_2$)-Leu-Gly-R

2a, R = OMe
b, R = NHMe

Results and Discussion

Synthesis of Peptides. The peptides **1a** and **1b** were synthesized by using a 3 + 4 coupling of their smaller

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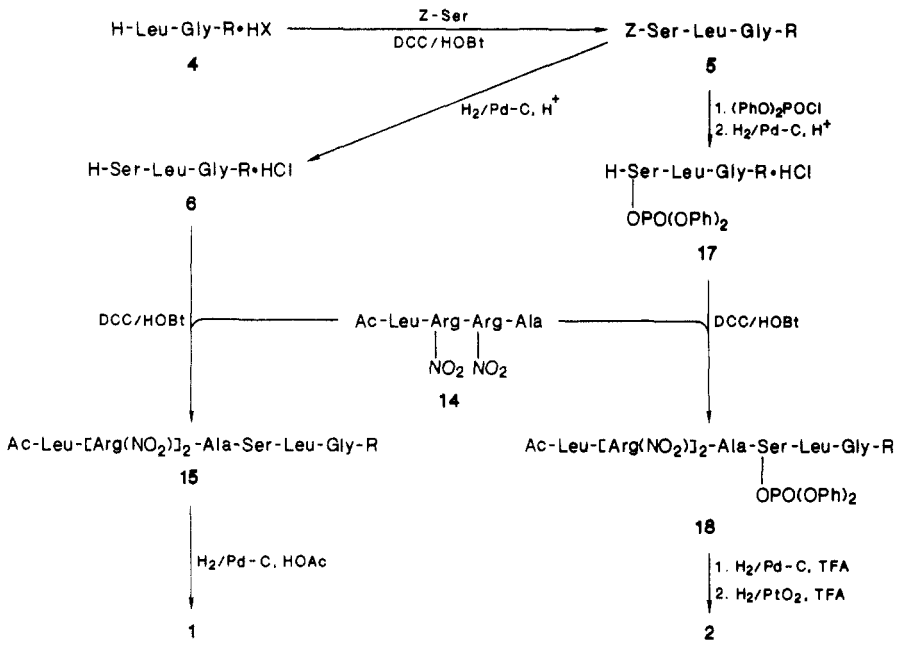
0022-3263/87/1952-1771$01.50/0

Scheme I[a]



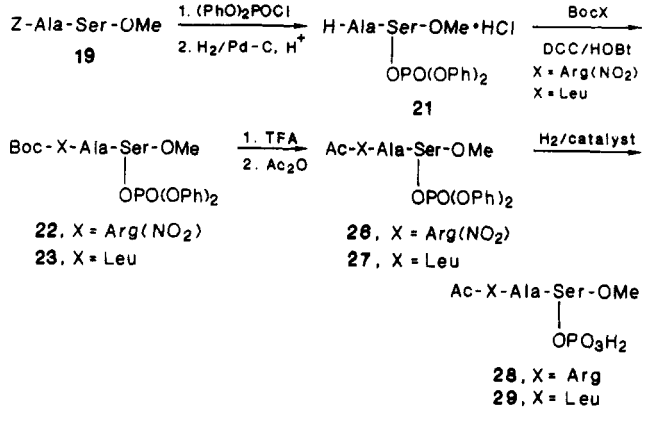
[a] (a) R = OMe; (b) R = NHMe.

peptide components (Scheme I). Z-Ser-Leu-Gly-OMe (**5a**) was synthesized by deprotection of Z-Leu-Gly-OMe[10] by catalytic hydrogenation to obtain H-Leu-Gly-OMe·HCl (**4a**), followed by subsequent coupling with Z-Ser using dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt).[11] The peptide Z-Ser-Leu-Gly-NHMe (**5b**) was obtained through the DCC/HOBt coupling of Boc-Leu·H_2O and Gly-NHMe·HCl,[12] deprotection of the resulting dipeptide with TFA, and coupling the trifluoroacetate salt (**4b**) with Z-Ser, again using DCC/HOBt. The tripeptides **5a** and **5b** were deprotected by catalytic hydrogenation to obtain the corresponding hydrochloride salts **6a** and **6b**.

Synthesis of the tetrapeptide Ac-Leu-Arg(NO_2)-Arg(NO_2)-Ala (**14**) was accomplished by building up the peptide chain from Ala-OBu-*t*·HCl with two successive couplings of Boc-Arg(NO_2) and a final coupling with Boc-Leu·H_2O, all by the DCC/HOBt method. The intermediary Boc peptide *tert*-butyl esters **7** and **9** were selectively deprotected at the amine termini with 85% formic acid[13] for the subsequent couplings. The formate salts so obtained were converted to their free amines (**8** and **10**) with 1 N NH_4OH prior to extraction into ethyl acetate. Following formic acid deprotection of the tetrapeptide **11**, the formate salt **12** was acetylated with acetic anhydride, with the acetylated product, **13**, being purified by plug filtration over silica gel. The *tert*-butyl ester was then cleaved with TFA to obtain the desired tetrapeptide **14**.

The tripeptides **6a** and **6b** were both coupled to **14** by the DCC/HOBt method. Following removal of the dicyclohexylurea (DCU) by filtration and evaporation of the dimethylformamide solvent, the heptapeptide products **15a** and **15b** were isolated by trituration with methanol. Reductive cleavage of the nitroarginine moieties in acetic acid using palladium on activated carbon afforded **1a** and **1b** as the diacetate salts.

Scheme II



Synthesis of Phosphopeptides. Attempts to effect the direct phosphorylation of **15a** with diphenylphosphochloridate[14,15] were unsuccessful. Therefore, the tripeptides **5a** and **5b** were each phosphorylated with diphenylphosphochloridate to obtain the diphenylphosphotripeptides **16a** and **16b**, which were deprotected at the amine termini by catalytic hydrogenation using palladium on activated carbon as the catalyst (Scheme I). The resultant hydrochloride salts **17a** and **17b** were each coupled to **14** using DCC/HOBt. The diphenylphosphoheptapeptides **18a** and **18b** were isolated in a similar fashion as **15a** and **15b**, triturating with 2:1 chloroform/methanol and methanol, respectively. Attempts to reductively cleave the nitro groups of **18a** by using palladium on activated carbon as the catalyst and acetic acid as the solvent (i.e., conditions successful in the deprotection of **15a** and **15b**) resulted in a complex mixture of products.

This critical deblocking reaction was studied further through the synthesis and evaluation of the model diphenylphosphopeptides Ac-Arg(NO_2)-Ala-(*O*-diphenylphospho)Ser-OMe (**26**) and Ac-Leu-Ala-(*O*-diphenylphospho)Ser-OMe (**27**), which were synthesized by the methodology outlined in Scheme II. Hydrogenation of

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Table I. Amino Acid Analysis and HPLC Retention Times of Heptapeptides

compd	Leu	Arg	Ala	Ser	Gly	t_R, min[a]
1a	2.12	2.06	1.00	0.94	1.09	11.0
1b	2.00	1.91	1.00	0.84	1.00	8.5
2a	2.05	2.03	1.00	0.79	1.05	7.0
2b	2.04	1.99	1.00	0.78	0.85	5.5
47	2.06	1.97	1.08	0.31[b]	1.00	10.5

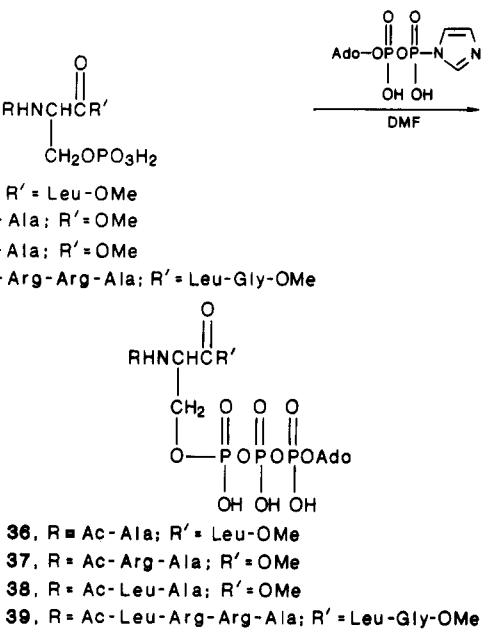
[a] See Experimental Section for HPLC conditions. [b] From Azy[23] moiety (see text).

26 in acetic acid using palladium on activated carbon as the catalyst resulted in complete loss of the original absorbance at 270 nm due to the nitro group and afforded a mixture of at least three components. One of these was purified by preparative TLC and identified on the basis of its proton NMR spectrum as the tripeptide Ac-Arg-Ala-Ala-OMe, which suggested a mechanism involving elimination of the diphenylphosphate moiety followed by reduction of the dehydroalanine-containing peptide. Successful reductive cleavage of the phenyl esters of **27** (a model compound in which leucine serves as a nonbasic, steric analogue of arginine) using Adams' catalyst in acetic acid effectively ruled out the participation of acetate in the elimination of **26** and strongly implicated a base-catalyzed process involving the reduced arginine moiety. This was further substantiated by the finding that the elimination–reduction pathway could be prevented by hydrogenating **26** in ethanol with 1 equiv of HCl, although reduction of the phenyl esters did not proceed to completion. Reduction of both the nitro groups and phenyl esters of **26** was accomplished with TFA as the solvent. It was therefore concluded that only those acids strong enough to completely protonate arginine could prevent this moiety from abstracting the α-proton of the serine residue (either inter- or intramolecularly) and hence initiating the elimination–reduction pathway. Since the use of TFA resulted in the complete reduction of **26**, it was the solvent of choice for the reduction of the diphenylphosphoheptapeptides **18a** and **18b**.[5] In addition, this acid was able to completely solubilize **18a** and **18b**, unlike the ethanol/HCl combination. Utilizing TFA as the solvent, the nitro groups and phenyl esters of both **18a** and **18b** were completely reduced in successive hydrogenations using palladium on activated carbon and platinum(IV) oxide as the catalysts, respectively, yielding the desired phosphoheptapeptides **2a** and **2b**.

Biochemical Evaluation of Synthetic Peptides. The heptapeptides **1a** and **1b** and the phosphoheptapeptides **2a** and **2b** all had satisfactory amino acid analyses and exhibited excellent purity on HPLC (Table I). The heptapeptides **1a** and **1b** were both competent substrates for the type II cAMP-dependent protein kinase from bovine cardiac muscle. The enzymatic product of the phosphorylation of **1a** was purified by gel filtration and preparative HPLC and was then found to co-elute on analytical HPLC with the chemically prepared phosphoheptapeptide **2a**. A preliminary determination of the kinetic parameters for the heptapeptide **1b** yielded K_m and V_{max} values of 12.7 μM and 8.0 μmol min^{-1} mg^{-1}, respectively, with a V_{max}/K_m ratio of 0.63 mg^{-1} L min^{-1}. The V_{max}/K_m ratio for **1b** is slightly lower than that reported for the completely deprotected kemptide.[2] These data show that peptides **1a** and **1b** bind well to the kinase and thus are good candidates for incorporation into γ-peptidyl esters of ATP as possible potent and specific kinase inhibitors.[9]

Synthesis of ATP γ-Peptide Esters. We have previously reported the synthesis of the ATP γ-peptide ester

Scheme III



of Ac-Ala-Ser-OMe.[9] This adduct, obtained through the carbonyldiimidazole-mediated coupling of ADP and the corresponding phosphodipeptide,[16] has no inhibitory effect on the type II catalytic subunit of cAMP-dependent protein kinase from bovine cardiac muscle (A. C. Nairn and P. Greengard, personal communication), presumably because the peptide portion lacks the arginine residues required for peptide substrate activity.[2] It was therefore desired to synthesize adducts containing longer peptide moieties, particularly those featuring arginine residues. The ATP γ-peptide ester of Ac-Ala-Ser-Leu-OMe (**36**, Scheme III) was successfully prepared by coupling the phosphopeptide Ac-Ala-(*O*-phospho)Ser-Leu-OMe (**35**) to ADP. This phosphopeptide was synthesized by standard methods: Z-Ser-Leu-OMe[17] was deprotected by catalytic hydrogenation and the resultant H-Ser-Leu-OMe·HCl (**30**) was coupled to Z-Ala by the DCC/HOBt method. Following phosphorylation of the serine hydroxyl group with diphenylphosphochloridate, the N-terminus was deprotected with HBr/AcOH and acetylated with acetic anhydride to obtain **34**. Reductive cleavage of the phenyl esters with Adams' catalyst in acetic acid afforded the desired phosphopeptide **35**.

The ultimate goal was to couple either of the phosphopeptides **2a** or **2b** to ADP to obtain an adduct in which the terminal phosphate of ATP is covalently esterified to the arginine-rich kemptide sequence. Following the successful coupling of **35** to ADP (in 77% yield), a precedent for coupling an arginine-containing phosphopeptide to ADP using the carbonyldiimidazole methodology was desired. The phosphotripeptide Ac-Arg-Ala-(*O*-phospho)-Ser-OMe·TFA (**28**), available from the hydrogenation model studies (Scheme II), was successfully coupled to ADP (Scheme III) to give **37**, but in very poor yield (8%). The phosphotripeptide Ac-Leu-Ala-(*O*-phospho)Ser-OMe (**29**), however, coupled to ADP to give **38** in 51% yield (Scheme III). This result implicated the reduced guanidinium of the arginine residue, rather than steric factors, as the main determinant in the low yield synthesis of the adduct **37**. Not surprisingly, an attempt to couple the phosphoheptapeptide **2a** (which contained two reduced

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Scheme IV

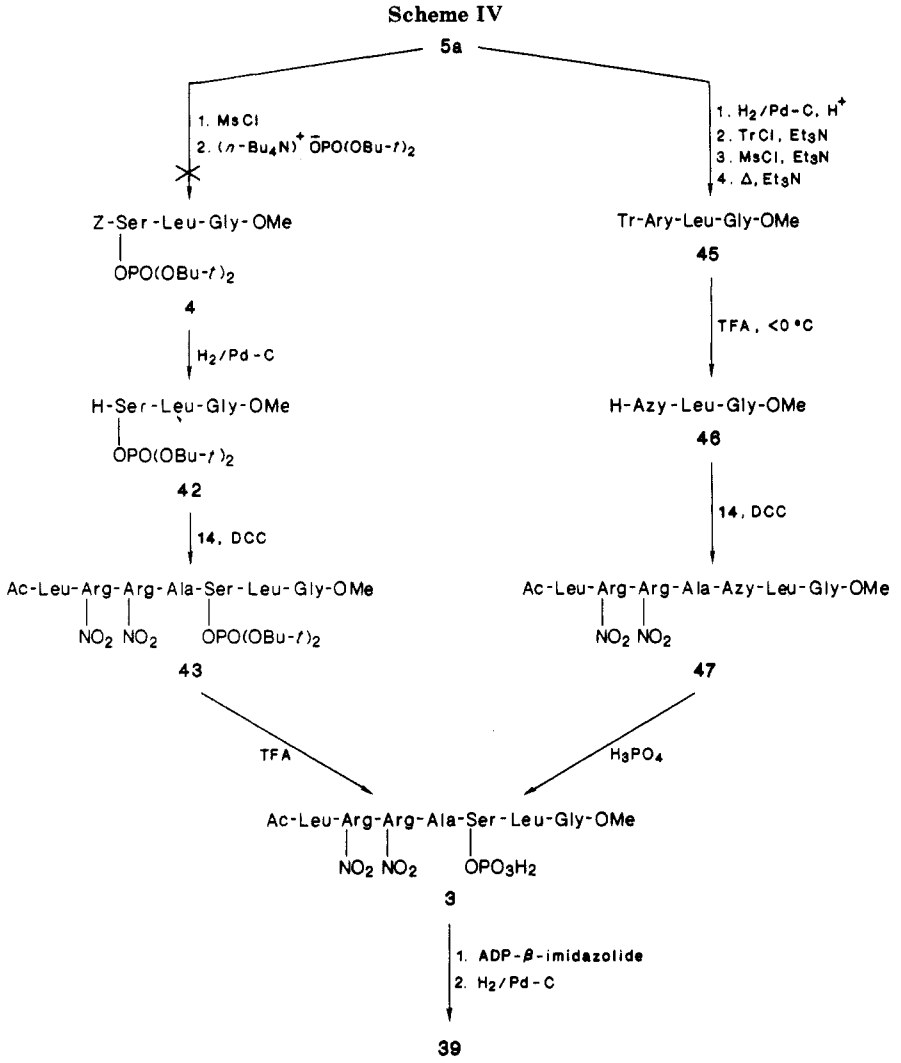

Table II. Chromatographic and ^{31}P NMR Data for ATP γ-Peptide Esters

ATP γ-peptide ester	DEAE elution concn (M NH_4HCO_3)	^{31}P chem shift (ppm)[a]		
		α-P	β-P	γ-P
36	0.085	−12.2	−23.6	−12.2
37	0.055	−12.1	−23.5	−12.1
38	0.080	−12.1	−23.6	−12.1

[a] In D_2O, pD 3, 2 mM EDTA. The α- and γ-signals appear as doublets (J = 20 Hz) while the β-signal appears as a triplet (J = 20 Hz).

guanidiniums as well as considerable steric bulk) to ADP in order to prepare **39** resulted in no reaction, suggesting that protection of the guanidiniums would be necessary to allow for effective coupling of arginine-containing phosphopeptides by the carbonyldiimidazole methodology. Chromatographic and ^{31}P NMR data for the synthetic ATP γ-peptide esters are summarized in Table II. Additional physical properties for these products are found in Table V.

Attempted Synthesis of a Nitroarginine-Containing Phosphopeptide. In view of the unsatisfactory results obtained in the carbonyldiimidazole reaction with phosphopeptides containing reduced guanidinium moieties, the phosphoheptapeptide Ac-Leu-Arg(NO_2)-Arg(NO_2)-Ala-(*O*-phospho)Ser-Leu-Gly-OMe (**3**) became a prime synthetic target. Use of **3** in the synthesis of ATP γ-peptide esters would involve the reductive cleavage of the nitro groups of the adduct following the carbonyldiimidazole coupling. Scheme IV outlines two proposed strategies for the synthesis of **3**. One method involves the mesylation of the tripeptide **5a** followed by displacement of the mesylate **40** with tetra-*n*-butylammonium di-*tert*-butyl phosphate[18] to obtain the di-*tert*-butylphosphopeptide **41**. The amine terminus of **41** would be deprotected by catalytic hydrogenation and coupled to the tetrapeptide **14** to obtain the di-*tert*-butylphosphoheptapeptide **43**. The phosphoheptapeptide **3** would then be obtained by cleavage of the *tert*-butyl esters of **43** with TFA. Our attempts to displace Z-(*O*-mesyl)Ser-Leu-Gly-OMe (**40**) with di-*tert*-butyl phosphate anion were unsuccessful. A single peptide component corresponding to approximately 60% of the starting material was purified from the reaction mixture by preparative TLC and tentatively identified through proton and carbon NMR, elemental analysis, and mass spectroscopy as Z-(3-chloro)Ala-Leu-Gly-OMe. By what mechanism this material is formed is not understood. Tetra-*n*-butylammonium di-*tert*-butyl phosphate has been reported to displace alkyl bromides to yield alkyl di-*tert*-butyl phosphates in excellent (>80%) yield.[18] However, our attempts to displace simple alkyl bromides with this reagent consistently yielded only 20–30% of the desired alkyl di-*tert*-butyl phosphates.

The second proposed route to **3** involved the phosphoric acid ring-opening reaction of the corresponding aziridine heptapeptide (**47**, Scheme IV).[19-21] The aziridine hepta-

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peptide **47** was synthesized from the tripeptide **5a**: Following deprotection by **5a** by catalytic hydrogenation and reaction of the N-terminal amine with trityl chloride to give **44**, the serine hydroxyl group was mesylated with methanesulfonyl chloride. The resultant mixture of mesylate and the aziridine tripeptide **45** was heated at reflux in tetrahydrofuran with 2 equiv of triethylamine to convert the remaining mesylate to the desired aziridine. The amine terminus of **45** was deprotected at −10 °C with TFA, and the deprotected aziridine tripeptide **46** was then coupled to **14** with DCC. HOBt was omitted from this coupling in order to avoid having to remove it from the crude reaction mixture by either extraction with dilute acids and bases or preparative thin layer chromatography on silica gel, conditions under which our acyl aziridine peptides decomposed. Following filtration of the DCU and removal of the solvent dimethylformamide under reduced pressure, the aziridine heptapeptide **47** product was isolated by trituration with ethyl acetate. The reaction was therefore run with a slight excess of **46**, such that all of **14** would react and any of the unreacted **46** could be removed during the trituration step. The aziridine heptapeptide **47** exhibited excellent purity on analytical TLC and HPLC. An amino acid analysis (Table I) gave the proper ratios of leucine, arginine, alanine, and glycine. The hydrolysis conditions (22 h, 109 °C) converted about 30% of the aziridine carboxylic acid residue to serine, with the remaining 70% presumably being converted to ring-opened products of unknown structures.

We then attempted to open the aziridine ring of **47** with 85% phosphoric acid to obtain **3**. Okawa et al.[19] reported that several sterically unhindered hydrophobic aziridine tripeptides could be converted to the corresponding phospho- and dibenzylphosphopeptides with 85% phosphoric and dibenzylphosphoric acid, respectively. The use of dibenzylphosphoric acid for the preparation of **3** by the aziridine methodology would be of no advantage, since the reducing conditions required for conversion of the resultant dibenzylphosphoheptapeptide to the phosphoheptapeptide would also reduce the nitro groups protecting the arginine guanidinium moieties. Using 85% phosphoric acid to open the aziridine ring of **47** presented a problem not encountered by Okawa et al.,[19] specifically that of isolating a very polar phosphopeptide from solvent amounts of phosphoric acid. To circumvent this, following treatment of the aziridine peptide **47** with 85% phosphoric acid, the reaction solution was brought to pH 7 by the addition of $Ba(OH)_2 \cdot 8H_2O$ (supernatant of a slurry, 3.1 g/100 mL) at 0 °C. This converted the phosphoric acid to insoluble barium phosphate, which could be easily removed by centrifugation. The barium salt of the product phosphopeptide was expected to be water-soluble at this pH on the basis of related experiments with the barium salt of **29**. However, a UV analysis showed that only 47% of the original material could be accounted for in the water-soluble fraction following lyophilization. The water-soluble fraction was shown to contain three components by HPLC, one of which co-eluted with the tetrapeptide **14**, indicating that a portion of **47** had been hydrolyzed at the aziridine amide bond. DEAE-cellulose chromatography using a 0.025–0.15 M NH_4HCO_3 gradient resolved one of the two remaining components of the water-soluble fraction, which corresponded to less than 10% of the material applied to the column. On the basis of its proton NMR (loss of methyl ester peak) and phosphorus NMR spectra, as well as its elution concentration from the DEAE-cellulose column (0.075 M NH_4HCO_3), the component was tentatively identified as Ac-Leu-Arg(NO_2)-Arg(NO_2)-Ala-(*O*-phospho)Ser-Leu-Gly, indicating that the barium hydroxide process had hydrolyzed the methyl ester of **47** or the ring-opened product (**3**). The remaining and unretained component was not positively identified, although it was not unreacted aziridine peptide, **47**, and a phosphorus NMR showed no resonances, indicating that it was not the desired product **3**.

In conclusion, we have successfully synthesized heptapeptide substrates for cAMP-dependent protein kinase as well as the corresponding phosphopeptide products, employing the techniques of solution-phase peptide synthesis. It is envisioned that these and related compounds will help facilitate the study of this important enzyme. Additionally, ATP γ-peptide esters incorporating portions of the peptide substrate sequence have been synthesized. The low reactivity of phosphopeptides **28** and **2a** with ADP-β-imidazolate establishes the necessity for arginine protection in this reaction and possibly (in the case of **2a**) the need to alleviate steric impediments. The synthetic approach described in this paper also should be applicable in the syntheses of related ATP γ-peptides containing peptide substrates for the insulin receptor tyrosine protein kinase.[22] Azy[23] peptides, such as heptapeptide **47**, in which an aziridine carboxylic acid is substituted for the serine residue, offer the potential as a useful electrophilic probe for the enzyme.

Experimental Section

Materials and Methods. Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. Lyophilizations were performed on Virtis automatic freeze dryer (Model No. 10-010). A Parr hydrogenation apparatus (Model No. 3911) was used for all hydrogenation reactions. Conductivity measurements were taken on a Radiometer Copenhagen conductivity meter (Type CDM3). A Sorvall General Laboratory centrifuge was used for all centrifugations. All NMR spectra were recorded on a Varian XL-200 NMR spectrophotometer at 200 MHz (proton) or 81 MHz (phosphorus, proton decoupled). Proton and phosphorus NMR chemical shifts are reported relative to tetramethylsilane and 85% phosphoric acid, respectively. All reagents and solvents were of the highest quality commercially available, and when necessary were further purified or dried by standard methods. Thin layer chromatography were performed on either silica gel (EM Reagents, No. 5775) or cellulose plates (Eastman, No. 13254). Thin layer chromatography systems were as follows: (A) silica gel, 4:1:1 butanol/acetic acid/water; (B) silica gel, 9:1 EtOAc/acetone; (C) silica gel, 20:1 $CHCl_3$/MeOH; (D) silica gel, 4:1 $CHCl_3$/MeOH; (E) silica gel, 9:1 $CHCl_3$/MeOH; (F) silica gel, 1:1 EtOAc/acetone; (G) silica gel, 9:1 acetone/EtOAc; (H) silica gel, 15:3:12:10 butanol/acetic acid/water/pyridine; (I) silica gel, 9:1 EtOAc/petroleum ether; (J) silica gel, 15:1 $CHCl_3$/MeOH; (K) silica gel, 2:1 $CHCl_3$/MeOH; (L) silica gel, 5:4:1 butanol/acetic acid/water; (M) silica gel, MeOH; (N) cellulose, 9:3:2:2:4 butanol/acetone/acetic acid/5% NH_4OH/water; (O) silica gel, 1:1 EtOAc/petroleum ether. Analytical HPLC was performed on either a Whatman Partisil PXS 10/25 ODS-1 or ODS-2 column, using an Altex solvent metering pump (Model 110A) and a Gilson Holochrome UV detector (Model HM). HPLC systems (column, wavelength, mobile phase, flow rate) were as follows: 1 (ODS-1, 260 nm, 65% MeOH/H_2O, 0.5 mL/min); 2 (ODS-2, 270 nm, 50% MeOH/H_2O, 1.0 mL/min); 3 (ODS-2, 270 nm, 65% MeOH/H_2O, 0.5 mL/min); 4 (ODS-2, 260 nm, 75% MeOH/H_2O, 1.0 mL/min); 5 (ODS-2, 260 nm, 65% MeOH/H_2O, 1.0 mL/min); 6 (ODS-2, 270 nm, 65% MeOH/H_2O, 1.0 mL/min); 7 (ODS-2, 260 nm, 60% MeOH/H_2O, 1.0 mL/min); 8 (ODS-1, 260 nm, 55% MeOH/H_2O,

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(22) Stadtmauer, L.; Rosen, O. M. *J. Biol. Chem.* **1986**, *261*, 10000–10005.
(23) The abbreviation "Azy" refers to an aziridine-2-carboxylic acid residue, as noted by Nakajima and co-workers (ref 20).

Table III. Physical Properties of Peptide Substrates and Their Intermediates

compd	method	yield, %	R_f (TLC system)	t_R, min (HPLC system)	product form (mp, °C)
H-Leu-Gly-OMe·HCl (**4a**)	A	98	0.62 (A)		glass
Z-Ser-Leu-Gly-OMe (**5a**)	B	66	0.42 (B), 0.31 (C)	7.4 (1)	microcrystalline (157–159)[a]
H-Ser-Leu-Gly-OMe·HCl (**6a**)	A	99	0.51 (A)		glass
Boc-Leu-Gly-NHMe	B[b]	64	0.65 (D), 0.79 (A)		glass[c]
H-Leu-Gly-NHMe·TFA (**4b**)	C	89	0.47 (A)		powder (168–169)
Z-Ser-Leu-Gly-NHMe (**5b**)	B[d]	28	0.61 (D), 0.61 (A)		amorphous (173–174)[a]
H-Ser-Leu-Gly-NHMe·HCl (**6b**)	A	98	0.45 (A)		glass
Boc-Arg(NO_2)-Ala-OBu-*t* (**7**)	B[b]	94	0.48 (E), 0.83 (A)	17.5 (2)	glass
H-Arg(NO_2)-Ala-OBu-*t* (**8**)	D	68	0.50 (A)		glass
Boc-[Arg(NO_2)]$_2$-Ala-OBu-*t* (**9**)	B	90	0.42 (F), 0.81 (A)	13.7 (3)	glass
H-[Arg(NO_2)]$_2$-Ala-OBu-*t* (**10**)	D	52	0.44 (A)		oil
Boc-Leu-[Arg(NO_2)]$_2$-Ala-OBu-*t* (**11**)	B	69	0.44 (F), 0.61 (G)	25.5 (3)	glass
H-Leu-[Arg(NO_2)]$_2$-Ala-OBu-*t*·HCO_2H (**12**)	D	89	0.51 (A)		powder[e]
Ac-Leu-[Arg(NO_2)]$_2$-Ala-OBu-*t* (**13**)	*f*	48	0.46 (D), 0.70 (A)	10.5 (3)	amorphous[e]
Ac-Leu-[Arg(NO_2)]$_2$-Ala (**14**)	C	96	0.51 (A), 0.70 (H)		powder[e]
Ac-Leu-[Arg(NO_2)]$_2$-Ala-Ser-Leu-Gly-OMe (**15a**)	E	83	0.79 (H), 0.62 (A)		amorphous (204–207)[g]
Ac-Leu-[Arg(NO_2)]$_2$-Ala-Ser-Leu-Gly-NHMe (**15b**)	E	59	0.70 (H), 0.43 (A)		amorphous[e]
Ac-Leu-Arg$_2$-Ala-Ser-Leu-Gly-OMe·2HOAc (**1a**)	F	96	0.69 (H)	11.0 (9)	powder[e,g]
Ac-Leu-Arg$_2$-Ala-Ser-Leu-Gly-NHMe·2HOAc (**1b**)	F	100	0.22 (A), 0.57 H)	8.5 (9)	powder[e,g]

[a] Satisfactory elemental analysis (±0.4 for C, H, and N). [b] THF reaction solvent. [c] Satisfactory elemental analysis [±0.4 for H and N, C 55.79 (calcd), 55.26 (found)]. [d] The crude product was salted back into EtOAc from the 1 M HCl wash and purified by preparative thin layer chromatography (silica gel GF, $CHCl_3$/MeOH (4:1) eluant). Further purification was effected by dissolving this material in warm MeOH and adding Et_2O to the cloud point. Analytically pure product slowly precipitated on standing at ambient temperature. [e] Wide melting point. [f] Prepared as described in Experimental Section. [g] Satisfactory amino acid analysis (Table I).

0.5 mL/min). HPLC system 9 was used to analyze peptides **1a**, **1b**, and **47**, and phosphopeptides **2a** and **2b** (column, Vydac C-18 (No. 218TP54); detector, Gilson Holochrome (Model HM); solution A, 95% 0.1 M $NaClO_4$ (pH 2.5), 5% CH_3CN; solution B, 40% 0.1 M $NaClO_4$ (pH 2.5), 60% CH_3CN; gradient, 20–80% solution B over 30 min; flow rate, 1.5 mL/min; wavelength, 207 nm).

Leu-Gly-OMe·HCl (4a). Palladium on activated carbon (2.4 g) was covered in a hydrogenation bottle with 5 mL of EtOH. To this mixture was added a solution of 23.465 g (69.8 mmol) of Z-Leu-Gly-OMe[10] in 150 mL of MeOH and 38.4 mL of (76.8 mmol) 2 N HCl. The mixture was hydrogenated 18 h at 30 psi. The catalyst was filtered off with Celite, and water was removed from the filtrate by several evaporations with MeOH under reduced pressure. The remaining solvent was removed under high vacuum, leaving the product as a slightly yellow glass (16.329 g, 68.4 mmol, 98%). For additional physical properties, see Table III.

Unless otherwise noted, all subsequent hydrogenations of carbobenzyloxy protecting groups were accomplished by this procedure, referred to as method A (Table III). Successful deprotection was ascertained by the absence of benzyl and aromatic resonances in the ^1H NMR of the product, as well as a ninhydrin positive reaction on TLC.

Z-Ser-Leu-Gly-OMe (5a). To a solution of 16.3 g (68.3 mmol) of **4a**, 16.339 g (68.3 mmol) of Z-Ser, 9.229 g (68.3 mmol) of 1-HOBt, and 7.51 mL (68.3 mmol) of *N*-methylmorpholine in 150 mL of dry DMF at −10 °C was added 16.898 g (81.9 mmol) of DCC. The reaction solution was stirred 1 h at −10 °C and 18 h at ambient temperature. The solvent was removed under reduced pressure, and EtOAc (150 mL) was added to the residue. The precipitated DCU was filtered off, and the EtOAc filtrate was washed with 1 M HCl (3 × 100 mL), 1 M $NaHCO_3$ (3 × 100 mL), and H_2O (3 × 100 mL) and then dried over Na_2SO_4. The solvent was removed under reduced pressure, leaving a white solid which was dissolved in a minimum volume of acetone. Additional DCU precipitated out and was filtered off. The solvent was again removed under reduced pressure, leaving a white solid which was crystallized from EtOAc–petroleum ether. The product was obtained as a white solid (18.965 g, 44.8 mmol, 66%). For additional physical properties, see Table III.

Unless otherwise noted, all subsequent peptide bond-forming reactions were carried out according to this procedure, referred to as method B (Table III). In cases where the free amine component was available (i.e., as opposed to the acid salt), no tertiary base was added to the reaction. When an acid-sensitive functionality (such as a Boc or *tert*-butyl ester group) was present, 10% citric acid was substituted for 1 M HCl in the workup step. Provided that the product peptide was soluble, EtOAc was the solvent normally used for the initial DCU filtration. In cases where the product peptide was insoluble in EtOAc, acetone was substituted for the initial filtration step. When acetone was used to filter the DCU from the crude reaction mixture, the filtrate was evaporated to dryness under reduced pressure, and the residue was dissolved in EtOAc containing either 1 M HCl or 10% citrate. Subsequent workup was as described above. The poor yield (28%) obtained for **5b** was due to loss of the product to the water layer during the aqueous–organic workup. Only a portion of **5b** could be salted back into ethyl acetate for purification by preparative thin layer chromatography. Isolation of **5b** by chromatography directly from the crude reaction mixture would likely afford a better yield. In all cases proton NMR was used to verify the structure of the desired products.

Leu-Gly-NHMe·TFA (4b). To 846 mg (2.8 mmol) of Boc-Leu-Gly-NHMe was added 10 mL of cold (4 °C) TFA. The resultant solution was allowed to warm to ambient temperature and was stirred for 1.5 h. The solvent was removed under reduced pressure. The oily residue solidified on trituration with Et_2O (3×), and the residual Et_2O was removed under reduced pressure. The product was obtained as a white powder (786 mg, 2.5 mmol, 89%). Additional physical properties are listed in Table III. Unless otherwise indicated, all subsequent acidolysis of Boc and *tert*-butyl ester protecting groups were accomplished by this procedure, referred to as method C (see Table III). Complete cleavage of the Boc and *tert*-butyl moieties was ascertained by the absence of the 9 H singlet at 1.4 ppm in the ^1H NMR. Boc group cleavage was also verified by a ninhydrin positive reaction on TLC.

Arg(NO_2)-Ala-OBu-*t* (8). To 8.275 g (18.5 mmol) of **7** was added 300 mL of cold (4 °C) 85% formic acid. The resultant solution was allowed to warm to ambient temperature and was stirred for 3.5 h. Following dilution with an equal volume of H_2O, the solvent was removed under reduced pressure at ambient temperature. The residue was partitioned between EtOAc (100 mL) and H_2O (100 mL), and the aqueous layer was separated and lyophilized. The resultant white powder was partitioned between EtOAc (100 mL) and 1 M NH_4OH (100 mL). The organic layer was removed and the aqueous layer (pH 11) was saturated with NaCl and extracted with EtOAc (4 × 100 mL). The combined EtOAc layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The product was obtained as a yellow glass (4.363 g, 12.6 mmol, 68%). Additional physical properties are listed in Table III.

Boc-Arg(NO_2)-Arg(NO_2)-Ala-OBu-*t* (**9**) and Boc-Leu-Arg-(NO_2)-Arg(NO_2)-Ala-OBu-*t* (**11**) were also selectively deprotected by using this procedure, referred to as method D (Table III). The cleavage product of **9**, Arg(NO_2)-Arg(NO_2)-Ala-OBu-*t* (**10**), required several additional extractions with EtOAc to obtain suf-

Table IV. Physical Properties of Phosphopeptide Products and Their Intermediates

compd	method	yield, %	R_f (TLC system)	t_R, min (HPLC system)	product form (mp, °C)	^{31}P chemical shift, ppm
Z-Ser[OP(O)(OPh)$_2$]-Leu-Gly-OMe (**16a**)	G	94	0.61 (B), 0.48 (I)	13.9 (4)	oil	−11.1[a]
Z-Ser[OP(O)(OPh)$_2$]-Leu-Gly-NHMe (**16b**)	G	87	0.39 (J)		oil	−11.6[a]
H-Ser[OP(O)(OPh)$_2$]-Leu-Gly-OMe·HCl (**17a**)	A	95	0.71 (A), 0.75 (K)		oil	
H-Ser[OP(O)(OPh)$_2$]-Leu-Gly-NHMe·HCl (**17b**)	A	98	0.68 (A), 0.78 (H)		oil	
Ac-Leu-[Arg(NO$_2$)]$_2$-Ala-Ser[OP(O)(OPh)$_2$]-Leu-Gly-OMe (**18a**)	E	65	0.81 (H), 0.66 (K)		amorphous (173–176)[b]	−11.1[c]
Ac-Leu-[Arg(NO$_2$)]$_2$-Ala-Ser[OP(O)(OPh)$_2$]-Leu-Gly-NHMe (**18b**)	E	34	0.60 (A), 0.81 (H)		amorphous[d]	−11.9[e]
Z-Ala-Ser[OP(O)(OPh)$_2$]-OMe (**20**)	G[f]	95	0.78 (E), 0.86 (A)	23.6 (5)	glass	−11.9[a]
H-Ala-Ser[OP(O)(OPh)$_2$]-OMe (**21**)	A	93	0.58 (A)		oil	
Boc-Arg(NO$_2$)-Ala-Ser[OP(O)(OPh)$_2$]-OMe (**22**)	B[g]	88	0.53 (E), 0.73 (A)	11.4 (6)	glass	−13.2[a]
Boc-Leu-Ala-Ser[OP(O)(OPh)$_2$]-OMe (**23**)	B[g]	99	0.63 (C), 0.67 (J)		glass	−12.1[a]
H-Arg(NO$_2$)-Ala-Ser[OP(O)(OPh)$_2$]-OMe·TFA (**24**)	C	96	0.53 (A)		powder[d]	
H-Leu-Ala-Ser[OP(O)(OPh)$_2$]-OMe·TFA (**25**)	C	86	0.71 (A), 0.77 (L)		powder (130–133)[b]	
Ac-Arg(NO$_2$)-Ala-Ser[OP(O)(OPh)$_2$]-OMe (**26**)	H	57	0.54 (D), 0.20 (E)	22.8 (2)	amorphous[d]	−11.6[e]
Ac-Leu-Ala-Ser[OP(O)(OPh)$_2$]-OMe (**27**)	H	97	0.65 (E), 0.82 (A)	14.5 (5)	oil	−12.6[a]
Ac-Arg-Ala-Ser[OP(O)(OH)$_2$]-OMe·TFA (**28**)	J	98	0.11 (A)		amorphous[d]	−1.8[i]
Ac-Leu-Ala-Ser[OP(O)(OH)$_2$]-OMe (**29**)	I	100	0.31 (A)		powder[d]	−0.5[i]
Ac-Leu-Arg$_2$-Ala-Ser[OP(O)(OH)$_2$]-Leu-Gly-OMe·2TFA (**2a**)	J	96	0.13 (A), 0.55 (H)	7.0 (9)	powder[d]	−0.2[i,j]
Ac-Leu-Arg$_2$-Ala-Ser[OP(O)(OH)$_2$]-Leu-Gly-NHMe·2TFA (**2b**)	J	92	0.05 (A), 0.40 (H)	5.5 (9)	powder[d]	0.3[i,j]

[a] In $CDCl_3$. [b] Melts with decomposition. [c] In $(CD_3)_2SO$. [d] Wide melting point. [e] In CD_3COD. [f] Starting peptide **19** was not completely soluble in pyridine, but dissolved during the course of the reaction. [g] THF reaction solvent. [h] In D_2O, 2 mM EDTA, pD 2.5. [i] In D_2O, pD 3.0. [j] Satisfactory amino acid analysis (Table I).

ficient yields from the NaCl-saturated, 1 M NH_4OH layer. The cleavage product of **11**, Leu-Arg(NO$_2$)-Arg(NO$_2$)-Ala-OBu-*t*·HCO_2H (**12**) was isolated directly as the formate salt following lyophilization. Physical properties are listed in Table III.

Ac-Leu-Arg(NO$_2$)-Arg(NO$_2$)-Ala-OBu-*t* (13). To a mixture of 2.830 g (4.0 mmol) of **12** and 848 mg (8.0 mmol) of dried Na_2CO_3 in 10 mL of dry MeOH at −10 °C was added via syringe 3.78 mL (40 mmol) of acetic anhydride. The reaction mixture was stirred 1 h at −10 °C and left in a refrigerator (4 °C) for 18 h. The solvent was removed under reduced pressure, leaving an oily residue. The desired product was purified by plug filtration over silica gel (4 × 10 cm column) using 4:1 $CHCl_3$/MeOH as the eluant. After pooling of the appropriate fractions, removal of the solvents under reduced pressure gave a slightly yellow oil (2.128 g). Following trituration with Et_2O (3 × 75 mL), the oil was dissolved in minimal warm MeOH. EtOAc was then added to turbidity, the mixture cooled at 4 °C, and the product slowly precipitated as an amorphous white solid (1.376 g, 1.9 mmol, 48%). Additional physical properties are listed in Table III.

Ac-Leu-Arg(NO$_2$)-Arg(NO$_2$)-Ala-Ser-Leu-Gly-OMe (15a). To a solution of 94 mg (0.29 mmol) of **6a**, 185 mg (0.29 mmol) of **14**, 39 mg (0.29 mmol) of 1-HOBt, and 0.32 mL (0.29 mmol) of *N*-methylmorpholine in 2 mL of dry DMF at −10 °C was added 65 mg (0.31 mmol) of DCC. The reaction solution was stirred 2 h at −10 °C and 18 h at ambient temperature. After standing in a freezer (−5 °C) for 20 h, the precipitated DCU was filtered off and washed with cold DMF. The solvent was removed under reduced pressure and MeOH (25 mL) was added to the oily yellow residue. The residue solidified on trituration and was allowed to stand under MeOH for 16 h at room temperature to allow for complete precipitation of the desired product. The white residue was again triturated with MeOH (2 × 25 mL). The residual MeOH was removed under reduced pressure, leaving the product as a white amorphous solid (220 mg, 0.24 mmol, 83%). Additional physical properties are listed in Table III.

The heptapeptide Ac-Leu-Arg(NO$_2$)-Arg(NO$_2$)-Ala-Ser-Leu-Gly-NHMe (**15b**) and the diphenylphosphoheptapeptides Ac-Leu-Arg(NO$_2$)-Arg(NO$_2$)-Ala-(*O*-diphenylphospho)Ser-Leu-Gly-OMe (**18a**) and Ac-Leu-Arg(NO$_2$)-Arg(NO$_2$)-Ala-(*O*-diphenylphospho)Ser-Leu-Gly-NHMe (**18b**) were also successfully synthesized by using this procedure, referred to as method E (Table III), with the exception that 2:1 $CHCl_3$/MeOH was used for the trituration and isolation of **18a**. See Table III for physical properties.

Ac-Leu-Arg-Arg-Ala-Ser-Leu-Gly-OMe·2AcOH (1a). To 209 mg (0.23 mmol) of **15a** was added 10 mL of glacial acetic acid. Complete solvation was accomplished by gentle heating on a steam bath. The solution was cooled to ambient temperature and transferred to a hydrogenation bottle containing 30 mg of palladium on activated carbon. The mixture was hydrogenated at 30 psi for 36 h, and the catalyst was filtered off with Celite. The filtrate (15 mL) was diluted with 1.5 volumes of H_2O and extracted with Et_2O (4 × 50 mL). The aqueous layer was passed through a Zetapore 0.45 μm filter and lyophilized, leaving the product as a fluffy white powder (210 mg, 0.22 mmol, 96%). For additional physical properties, see Table III.

The heptapeptide Ac-Leu-Arg-Arg-Ala-Ser-Leu-Gly-NHMe·2AcOH (**1b**) was prepared from **15b** by the same procedure, referred to as method F (Table I). Heptapeptides **1a** and **1b** were characterized by proton NMR, TLC, HPLC, and amino acid analysis (see Tables I and III).

Z-(*O*-diphenylphospho)Ser-Leu-Gly-OMe (16a). To a solution of 847 mg (2.0 mmol) of **5a** in 4 mL of dry pyridine at −10 °C was added via syringe 0.5 mL (2.4 mmol) of diphenylphosphochloridate. A white precipitate of pyridinium hydrochloride appeared after 5 min. The reaction mixture was stirred 2.5 h at −10 °C and was left in a refrigerator (4 °C) for 18 h. The mixture was then diluted with 1 mL of H_2O (precipitate dissolved) and stirred an additional 30 min at room temperature. The solution was diluted with $CHCl_3$ (75 mL) and washed with 1 M HCl (3 × 50 mL), 1 M $NaHCO_3$ (3 × 50 mL), and H_2O (3 × 50 mL). After drying over Na_2SO_4, the solvent was removed under reduced pressure, leaving the product as a red-brown oil (1.231 g, 1.9 mmol, 94%). For additional physical properties, see Table IV.

Unless otherwise noted, all subsequent phosphorylations of serine-containing peptides using diphenylphosphochloridate were carried out according to this procedure, referred to as method G (Table IV). Successful diphenylphosphorylation was confirmed by the presence of a singlet in the −9.0 to −12.5 ppm range in the phosphorus NMR. In the case of **32**, crystallization was effected from EtOAc–petroleum ether.

Ac-Arg(NO$_2$)-Ala-(*O*-diphenylphospho)Ser-OMe (26). To a solution of 2.205 g (3.0 mmol) of **24** in 15 mL dry pyridine at −10 °C was added via syringe 2.83 mL (30 mmol) of acetic anhydride. The reaction solution was stirred 1 h at −10 °C and was left in a refrigerator (4 °C) for 18 h. The solvent was removed under reduced pressure, and the oily brown-red residue was

Table V. Physical Properties of ATP-γ-Peptidyl Esters and Their Intermediates

compd	method	yield, %	R_f (TLC system)	t_R, min (HPLC system)	product form (mp, °C)
H-Ser-Leu-OMe·HCl (**30**)	A	99	0.41 (M)		glass
Z-Ala-Ser-Leu-OMe (**31**)	B[a]	64	0.38 (B)	15.0 (7)	microcrystalline (126–128)[b]
Z-Ala-Ser[OP(O)(OPh)$_2$]-Leu-OMe (**32**)	G	92	0.65 (B), 0.71 (H)	16.4 (4)	microcrystalline (101–103)[b]
H-Ala-Ser[OP(O)(OPh)$_2$]-Leu-OMe·HBr (**33**)	c	90	0.70 (H)		oil
Ac-Ala-Ser[OP(O)(OPh)$_2$]-Leu-OMe (**34**)	H	85	0.15 (I), 0.53 (E)	20.0 (5)	powder[d]
Ac-Ala-Ser[OP(O)(OH)$_2$]-Leu-OMe (**35**)	I	100	0.35 (A)		powder[d]
ATP-γ-Ser-Leu-OMe | Ac-Ala (**36**)[e]	K	77	0.75 (N)		glass
ATP-γ-Ser-OMe | Ac-Arg-Ala (**37**)[e]	K	8	0.46 (N)		glass
ATP-γ-Ser-OMe | Ac-Leu-Ala (**38**)[e]	K	51	0.65 (N)		powder[d]

[a] THF reaction solvent. [b] Satisfactory elemental analysis (±0.4 for C, H, and N). This product was crystallized from EtOAc–petroleum ether. [c] 31% HBr/HOAc, initially at 0 °C, then to ambient temperature for 2 h.[9] [d] Wide melting point. [e] See Scheme III for structure of compound.

partitioned between EtOAc (100 mL) and 1 M HCl (150 mL). The aqueous layer was removed and the organic layer was washed with 1 M HCl (2 × 50 mL), 1 M $NaHCO_3$ (3 × 50 mL), and H_2O (3 × 50 mL) and then dried over Na_2SO_4. The solvent was removed under reduced pressure, leaving the product as a light brown solid residue (1.136 g, 1.7 mmol, 57%). Additional physical properties are listed in Table IV.

Unless otherwise indicated, all subsequent acetylations were carried out according to this procedure, referred to as method H (Table IV). Successful acetylation was confirmed by the presence of a 3 H singlet at 2.0 ppm in the proton NMR.

Ac-Leu-Ala-(*O*-phospho)Ser-OMe (29). In a hydrogenation bottle, 35 mg of platinum(IV) oxide was covered with 2 mL of glacial acetic acid. To this mixture was added a solution of 150 mg (0.26 mmol) of **27** in 5 mL of glacial acetic acid. The mixture was hydrogenated at 30 psi for 48 h, and the catalyst was filtered off with Celite. The acetic acid filtrate was diluted with two volumes of H_2O and extracted with Et_2O (3 × 40 mL). The aqueous layer was lyophilized, leaving the product as a fluffy white powder (112 mg, 0.26 mmol, 100%). Additional physical properties are listed in Table IV.

The peptide Ac-Ala-(*O*-phospho)Ser-Leu-OMe (**35**) was synthesized from **34** by using the identical procedure, referred to as method I (Table IV). Successful reductive cleavage of the phenyl esters was confirmed by lack of aromatic absorbances in the proton NMR. See Table V for physical properties.

Ac-Arg-Ala-(*O*-phospho)Ser-OMe·TFA (28). In a hydrogenation bottle 50 mg of palladium on activated carbon was covered with 2 mL of TFA. To this mixture was added a solution of 284 mg (0.43 mmol) of **26** in 5 mL of TFA. The mixture was hydrogenated at 30 psi for 36 h, and the catalyst was filtered off with Celite. The solvent was removed under reduced pressure and the oily brown residue solidified on trituration with Et_2O (3 × 40 mL). The residual ether was removed under reduced pressure, leaving a brown solid (420 mg), R_f (system A) 0.60. Complete reduction of the nitro group was confirmed by loss of absorbance at 270 nm. The residue was redissolved in 5 mL of TFA and transferred to a hydrogenation bottle containing 70 mg of platinum(IV) oxide. The mixture was hydrogenated 36 h at 30 psi, after which time an additional 30 mg of platinum(IV) oxide was added. The mixture was further hydrogenated at 30 psi for 36 h, and the catalyst was filtered off with Celite. The solvent was removed under reduced pressure, leaving a blackish brown residue, which was triturated with Et_2O (3 × 40 mL). The residue was then dissolved in H_2O and filtered through a 0.45-μm Zetapore filter. The filtrate was lyophilized, leaving the product as an amorphous white solid (245 mg, 0.42 mmol, 98%). Additional physical properties are listed in Table IV.

The phosphoheptapeptides Ac-Leu-Arg-Arg-Ala-(*O*-phospho)Ser-Leu-Gly-OMe·2TFA (**2a**) and Ac-Leu-Arg-Arg-Ala-(*O*-phospho)Ser-Leu-Gly-NHMe·2TFA (**2b**) were prepared from **18a** and **18b**, respectively, by the same procedure, referred to as method J. Complete reduction was verified by lack of absorbance at 270 nm (nitro groups) and loss of aromatic resonances in the proton NMR (phenyl esters). Additional physical properties are listed in Tables I and IV.

ATP γ-Peptide Ester of Ac-Ala-Ser-Leu-OMe (36). (a) Tri-*n*-butylammonium Ac-Ala-(*O*-phospho)Ser-Leu-OMe. A solution of 130 mg (0.30 mmol) of **35** in 2 mL of 3:1 MeOH/H_2O was applied by gravity to a 1.3 × 25 cm column of pyridinium Dowex (AG 50W-X8, 100-200 mesh, 1.7 mequiv/mL resin bed). After elution with the same solvent at a flow rate of 1.0 mL/min, the appropriate fractions (detected by UV absorbance) were pooled, and to this solution was added 0.072 mL (0.30 mmol) of tri-*n*-butylamine. The solvents were removed under reduced pressure, and water was removed by evaporation with pyridine (3 × 10 mL) and DMF (3 × 10 mL). The product was obtained as a yellow oil.

(b) Tri-*n*-butylammonium ADP. To a mixture of 42 mg (0.1 mmol) of ADP in 10 mL of dry 1:1 MeOH/pyridine at room temperature was added 48 μL (0.2 mmol) of tri-*n*-butylamine. The reaction mixture was stirred 0.5 h at ambient temperature, whereby it became a clear solution. The solvents were removed under reduced pressure and moisture was removed by evaporation with pyridine (2 × 10 mL) and DMF (2 × 10 mL). The product was obtained as a yellow oil.

(c) Coupling Reaction and Purification. To a solution of the freshly prepared tri-*n*-butylammonium ADP (0.1 mmol) in 1 mL of dry DMF at room temperature was added via syringe a solution of 82 mg (0.5 mmol) of carbonyldiimidazole in 1.5 mL of dry DMF. The reaction solution was stirred 4 h at ambient temperature, after which time quantitative conversion to ADP imidazolate was ascertained by thin layer chromatography (R_f(adduct, system N) 0.37, R_f(ADP, system N) 0.20). The excess carbonyldiimidazole was consumed by the addition of 0.033 mL (0.8 mmol) of dry MeOH. A solution of the freshly prepared tri-*n*-butylammonium Ac-Ala-(*O*-phospho)Ser-Leu-OMe (0.30 mmol) in 5 mL of dry DMF was then added via syringe, and the reaction solution was stirred 18 h at room temperature. After dilution with an equal volume of MeOH, the solvents were removed under reduced pressure. Addition of H_2O (10 mL) resulted in an oily precipitate, which was removed by centrifugation. The aqueous solution was diluted with H_2O to a conductance equal to that of 0.025 M NH_4HCO_3 and was loaded by gravity on a 0.9 × 30 cm column of DEAE-cellulose (DE-52, 1 mequiv/g dry weight) at 4 °C. After washing with 30 mL of 0.025 M NH_4HCO_3, the product was eluted with a linear gradient of 0.025–0.15 M NH_4HCO_3 (volume = 500 mL, pH 8, flow rate = 0.5 mL/min). Fractions (volume = 10 mL) were analyzed by UV absorbance. The product (major component) eluted at [NH_4HCO_3] = 0.085 M, and the appropriate fractions were pooled and lyophilized. Three lyophilizations were necessary to remove the residual NH_4HCO_3. The product was obtained as a colorless glass (64 mg, 0.077 mmol, 77%). Additional physical properties are found in Tables II and V.

The ATP γ-peptide ester of Ac-Arg-Ala-Ser-OMe·TFA (**37**) and the ATP γ-peptide ester of Ac-Leu-Ala-Ser-OMe (**38**) were syn-

Table VI.[a] Physical Properties of Aziridinyl Peptides and Related Intermediates (Scheme IV)

compd	yield, %	R_f (TLC system)	t_R, min (HPLC system)	product form (mp, °C)
Z-Ser-(OMs)Leu-Gly-OMe (40)	65	0.47 (C)8, 0.62 (B)		amorphous (150–151)[b]
Tr-Ser-Leu-Gly-OMe (44)	96	0.15 (O)	16.7 (8)	glass
Tr-Azy-Leu-Gly-OMe (45)	85	0.29 (O)	15.0 (4)	powder[c,d]
H-Azy-Leu-Gly-OMe (46)	85	0.59 (A)		oil
Ac-Leu-[Arg(NO_2)]$_2$-Ala-Azy-Leu-Gly-OMe (47)	92	0.55 (A), 0.80 (H)	10.5 (9)	powder[d-f]

[a] Compounds prepared as described in Experimental Section. [b] Satisfactory elemental analysis (±0.4 for C, H, and N). [c] Satisfactory elemental analysis (±0.4 for C, H, and N) for monohydrate. [d] Wide melting point. [e] Satisfactory elemental analysis (±0.4 for C, H, and N) for dihydrate. [f] Satisfactory amino acid analysis (Table I).

thesized by the same procedure, referred to as method K (Table V). In both cases, the coupling reaction solution was stirred for 72 h at ambient temperature rather than 18 h. Prior to purification of **37** on DEAE-cellulose, the aqueous solution (pH 5) was brought to pH 8 by the addition of 10% NH_4OH at 4 °C. Elution concentrations from DEAE-cellulose for **37** and **38** were 0.055 and 0.080 M NH_4HCO_3, respectively. The ATP γ-peptide esters **36**, **37**, and **38** all showed phosphorus and proton NMR spectra consistent with their assigned structure. Physical properties are listed in Tables II and V.

Z-(*O*-mesyl)Ser-Leu-Gly-OMe (40). To a solution of 2.47 g (5.8 mmol) **5a** in 15 mL of dry pyridine at −10 °C was added via syringe 0.494 mL (6.4 mmol) of methanesulfonyl chloride. The reaction solution was stirred 1 h at −10 °C and was left in a refrigerator (4 °C) for 18 h. The solution was partitioned between EtOAc (200 mL) and 1 M HCl (100 mL). The aqueous layer was removed, and the organic layer was washed with 1 M HCl (2 × 100 mL), 1 M $NaHCO_3$ (3 × 100 mL), and H_2O (3 × 100 mL) and then dried over Na_2SO_4. The solvent was removed under reduced pressure, leaving a white solid (2.824 g), which was reprecipitated from EtOAc. The product was obtained as a white amorphous solid (1.892 g, 3.8 mmol, 65%). Successful mesylation was confirmed by the presence of the methanesulfonyl singlet at 3.0 ppm in the proton NMR. For additional physical properties, see Table VI.

Tr-Ser-Leu-Gly-OMe (44). To a solution of 3.258 g (10 mmol) of **6a** and 2.78 mL (20 mmol) of triethylamine in 50 mL of dry $CHCl_3$ at −10 °C was added dropwise a solution of 2.788 g (10 mmol) of triphenylchloromethane in 10 mL of dry $CHCl_3$. The reaction solution was stirred 4 h at −10 °C and left in a refrigerator (4 °C) for 18 h. The $CHCl_3$ solution was washed with H_2O (3 × 50 mL), 10% citric acid (3 × 50 mL), and H_2O (3 × 50 mL) and then dried over Na_2SO_4. The solvent was removed under reduced pressure, leaving the product as a slightly yellow glass (5.091 g, 9.6 mmol, 96%). Additional physical properties are listed in Table VI.

Tr-Azy-Leu-Gly-OMe (45). To a solution of 5.091 g (9.6 mmol) of **44** and 2.0 mL (14.3 mmol) of triethylamine in 15 mL of dry CH_2Cl_2 at −10 °C was added via syringe 0.82 mL (10.5 mmol) of methanesulfonyl chloride. The reaction solution was stirred 4 h at −10 °C and was left in a refrigerator for 18 h. The CH_2Cl_2 solution was washed with H_2O (3 × 50 mL), 10% citric acid (3 × 50 mL), saturated $NaHCO_3$ (2 × 50 mL), and H_2O (2 × 50 mL) and then dried over Na_2SO_4. The solvent was removed under reduced pressure, leaving an oily mixture (4.989 g) consisting of the *O*-mesyl and aziridine-containing peptides. The oil was dissolved in 50 mL of dry THF and 2.67 mL (19.2 mmol) of triethylamine was added. The solution was heated at reflux for 48 h. Following cooling to ambient temperature, the solvent was removed under reduced pressure, and the resultant oily brown residue was dissolved in EtOAc (75 mL). The EtOAc solution was washed with 10% citric acid (3 × 50 mL), 1 M $NaHCO_3$ (3 × 50 mL), and H_2O (3 × 50 mL). The organic layer was then dried over Na_2SO_4, and the solvent was removed under reduced pressure, leaving an oil which solidified on trituration with petroleum ether (3 × 50 mL). The product was obtained as a light brown powdery solid (4.158 g, 8.1 mmol, 85%). Successful ring closure was confirmed by identification of the characteristic resonances due to aziridine protons in the NMR ($CDCl_3$): δ 1.48 (m, 1 H, αCH), 2.00 (m, 2 H, βCH_2), partially obscured by leucine γCH and βCH_2 protons. Additional physical properties are found in Table VI.

Azy-Leu-Gly-OMe (46). To a solution of 549 mg (1.0 mmol) of **45** in 5 mL of dry 1:1 $CHCl_3$/MeOH at −30 °C was added dropwise over 20 min 5 mL of cold (4 °C) trifluoroacetic acid. The solution was stirred at −10 °C for 1.5 h and the solvents were removed under reduced pressure at ambient temperature. The oily yellow residue was partitioned between Et_2O (50 mL) and H_2O (50 mL). The aqueous layer was brought to pH 8 with saturated $NaHCO_3$ solution and saturated with NaCl and then washed with EtOAc (3 × 75 mL). The combined EtOAc layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The product was obtained as a yellow oil (231 mg, 0.85 mmol, 85%). Successful detritylation without ring opening was confirmed by the absence of aromatic resonances and presence of aziridine resonances in the proton NMR ($CDCl_3$, D_2O): δ 1.85 and 1.90 (2 m, 2 H, βCH_2), 2.56 (m, 1 H, αCH). Additional physical properties are listed in Table VI.

Ac-Leu-Arg(NO_2)-Arg(NO_2)-Ala-Azy-Leu-Gly-OMe (47). To a solution of 493 mg (0.76 mmol) of **14** and 223 mg (0.82 mmol) of **46** in 3 mL of dry DMF at −10 °C was added 160 mg (0.77 mmol) of DCC. The reaction solution was stirred 1.5 h at −10 °C and 18 h at ambient temperature. After standing an additional 18 h in a freezer (−5 °C), the precipitated DCU was filtered off and rinsed with cold (4 °C) DMF. The solvent was removed under reduced pressure at room temperature, and the resultant yellow oil solidified on trituration with EtOAc (5 × 50 mL). The residual EtOAc was removed under reduced pressure. The product was obtained as a white powdery solid (626 mg, 0.70 mmol, 92%). The presence of the acyl aziridinyl moiety in the product was confirmed by the ^1H NMR spectrum [$(CD_3)_2SO$]: δ 2.37 and 2.62 (2 m, 2 H, βCH_2), 3.15 (m, 1 H, αCH). Additional physical properties appear in Tables I and VI.

Acknowledgment. This work was supported by a grant from the U.S. Public Health Service, National Institutes of Health, CA-28097. We thank Drs. E. Thomas Kaiser and Michael Doughty of Rockefeller University for performing the protein kinase assays and Mr. Don Guarino of the New York State Department of Health for the amino acid analyses. We are grateful to Dr. Anthony DeStefano of Norwich Eaton Pharmaceuticals, Inc. for the mass spectral analysis and to Sharon Derrick for typing the manuscript.