Table I. Selective Monoesterification of Diols[a]

diol	catalyst salt	catalyst mmol	time, h	yield and recovery, % monoester	diester	alcohol
ethylene glycol[b]	$NaHSO_4$	0.15	5	78	3	14
1,4-butanediol	$Ce(SO_4)_2$	0.063	4	68	0	30
1,4-butanediol	$Ce(SO_4)_2$	0.063	6	78	4	15
1,4-butanediol	$NaHSO_4$	0.125	6	81	5	11
1,4-butanediol[c]	$NaHSO_4$	0.1	2.2	92	2	7
1,4-butanediol[d]	$NaHSO_4$	0.125	24	94	1	7
1,4-butanediol[d]	$NaHSO_4$	0.125	33	97	2	0
1,5-pentanediol	$NaHSO_4$	0.125	5	78	3	19
1,6-hexanediol	$Ce(SO_4)_2$	0.083	5	80	6	15
2,5-hexanediol[e]	$NaHSO_4$	0.01	6	69	7	23

[a] A diol (1 mmol) and a supported salt (3 mmol/g of SiO_2) were heated at 50 °C in ethyl acetate–hexane (1:4) (15 mL). Yields were measured by GLC. [b] Ethyl acetate:hexane = 1:3. [c] This run was done at 70 °C in methyl propionate–hexane (1:4). [d] This run was done at 60 °C in methyl isobutyrate–hexane (1:4). [e] This run was done at room temperature in ethyl formate–hexane (1:4).

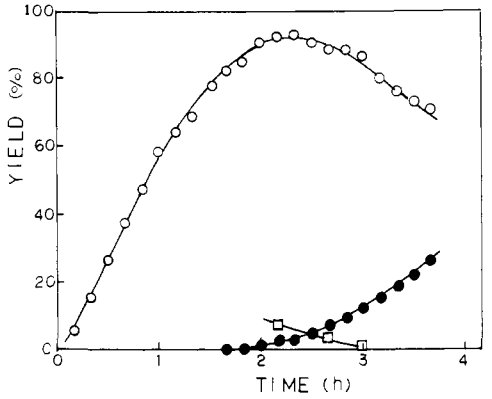

Figure 1. Yields and recoveries vs reaction time. 1,4-Butanediol (1 mmol) and $NaHSO_4$–SiO_2 (0.1 mmol) were heated in methyl propionate–hexane (1:4) at 70 °C: the monoester, O; the diester, ●; the diol, □.

be explained by the following presumptions: (1) only the alcohols adsorbed on the catalyst surface reacted; (2) as long as the diol, which is more polar and more apt to be adsorbed than the monoester, remained, it reacted preferentially; (3) the monoester was adsorbed and reacted after most of the diol had been consumed; and (4) the reactivity per hydroxyl group is alike both in the diol and in the monoester as long as these compounds are adsorbed. When the ratio of hexane gradually increased in the acylation of 1,4-butanediol in the methyl isobutyrate–hexane mixture, the yields of the monoester at 2% yield of the diester rose at first, showed the maximum value (97%) at the hexane:methyl isobutyrate ratio of 4:1, and then began to decrease (Figure 2). The reason why the selectivity to the monoester depends on the polarity of the mixed solvent may be explained by the following assumptions: (1) when the polarity of the mixed solvent was high, there was little selectivity in the adsorption on the catalyst between the diol and the monoester; (2) when it was adjusted adequately by the addition of hexane, only the diol was selectively adsorbed and acylated; and (3) when it decreased further, the selectivity decreased because the monoester too was adsorbed. The R_f values of the substances on silica gel TLC plates developed by the mixed solvents are also shown in Figure 2 for reference. Using smaller amounts of the catalysts generally raised the selectivity. This may be due to reduced adsorption of the monoester in the presence of small amounts of the diol. It is inferred that the surface of silica gel forms a "reaction field" where reagents and substrates are accumulated by adsorption and binds more polar substances in preference to less polar ones. This inference suggests that such a selective reaction as this esterification generally occurs when the polarity decreases successively from starting materials to the final products. This suggestion is supported by the preliminary result that 1,*n*-diols are selectively monoprotected by pyranyl ether formation using dihydropyran in the presence of some kind of

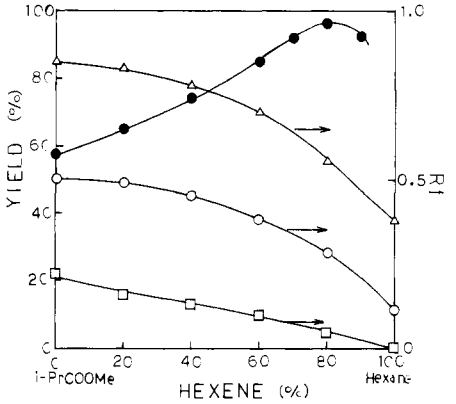

Figure 2. Yields of the monoester (●) at the 2% yield of the diester and R_f values of the diester (△), the monoester (O), and the diol (□) vs solvent composition. 1,4-Butanediol (1 mmol) and $NaHSO_4$–SiO_2 (0.125 mmol) were heated at 60 °C in the methyl isobutyrate–hexane mixture (15 mL). The R_f values were obtained by the use of silica gel 60 TLC plates (Merck).

$M_m(SO_4)_n$–SiO_2.

Registry No. $NaHSO_4$, 7681-38-1; $Ce(SO_4)_2$, 13590-82-4; ethylene glycol, 107-21-1; 1,4-butanediol, 110-63-4; 1,5-pentanediol, 111-29-5; 1,6-hexanediol, 629-11-8; 2,5-hexanediol, 2935-44-6; ethyl acetate, 141-78-6; methyl propionate, 554-12-1; methyl isobutyrate, 547-63-7; ethyl formate, 109-94-4; ethylene glycol monoacetate, 542-59-6; ethylene glycol diacetate, 111-55-7; 4-hydroxybutyl acetate, 35435-68-8; 1,4-butanediol diacetate, 628-67-1; 4-hydroxybutyl propionate, 33498-48-5; 1,4-butanediol dipropionate, 1572-92-5; 4-hydroybutyl isobutyrate, 123641-46-3; 1,4-butanediol diisobutyrate, 1572-74-3; 5-hydroxypentyl isobutyrate, 123641-47-4; 1,5-pentanediol diisobutyrate, 123641-48-5; 6-hydroxyhexyl isobutyrate, 101830-67-5; 1,6-hexanediol diisobutyrate, 101830-68-6; 2,5-hexanediol monoformate, 123674-08-8; 2,5-hexanediol diformate, 123641-49-6.

Synthesis of a Cyclic Phosphopeptide Containing a Phosphodiester Linkage

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Although protein phosphorylation is recognized as a major regulatory process[1,2] mediated by protein kinases, the molecular basis of changes induced by phosphorylation is virtually unknown. However, at least two examples in the recent literature may contribute to deepen our insight into this important posttranslational modification of peptides and proteins. In the first example,[3] the refined crystal structures of glycogen phosphorylase b and a, which differ only in one phosphorylated serine residue at position 14, were compared. This important study[3] may add to a further understanding of control by phosphorylation. For this reason, among others, we are interested in the synthesis and structure of phospho amino acids and phosphopeptides.[4,5] In the second

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Table I. NMR Data[a] of the Cyclic Phosphopeptide **5** in CD_3OD, T = 306 K

amino acid residue	atom no.[b]	^{1}H NMR			^{13}C NMR		^{31}P NMR
		δ, ppm	J_{HH}, Hz	J_{PH}, Hz	δ, ppm	J_{PC}, Hz	
serine	1				171.5[c]		
	2	4.36	2.9[d]	2.6	55.1	4.4	
	3	4.07	11.3[e]	10.8	67.1	5.9	
		4.30		7.8			
glycine	1				171.6[c]		
	2	3.75	17.0[e]		44.1		
		4.15					
threonine	1				174.2[c]		
	2	4.18[f]	2.2[d]		61.4	8.8	
	3	4.89	6.4[d]	2.4	75.8	4.4	
	4	1.49			18.4		
$N(H)CH_3$		2.76			26.6		
Boc		1.51			28.6		
					81.2		
					157.9		
P							−0.39

[a] ^{1}H NMR spectra were recorded on a Bruker WM 300-MHz apparatus interfaced with an ASPECT 2000 computer. ^{13}C and ^{31}P NMR spectra were measured on a JEOL JEC 980 B spectrometer, operating at 50.3 MHz and 80.7 MHz. TMS was used as internal and 85% H_3PO_4 as external standard. [b] Numbering of the amino acid carbons is according to IUPAC recommendations.[22] [c] The C^1 signals could not be assigned to the individual C^1's. [d] Vicinal coupling constant. [e] Geminal coupling constant. [f] Partly covered by signal due to glycine proton resonating at 4.15 ppm.

example the presence of an earlier proposed[6] phosphodiester linkage between hydroxyl groups of serine and threonine in the protein flavodoxin from *Azotobacter* was corroborated by Live and Edmondson[7] using ^{1}H–^{31}P two-dimensional NMR. Although this appears to be the only example so far of a possible phosphodiester linkage between a serine and a threonine residue in a protein, it might well be that a phosphodiester linkage serves a similar purpose as a disulfide linkage, i.e., to retain or stabilize the structure of a protein.[8]

The presence of such a potentially structurally important phosphodiester linkage aroused our interest in the synthesis of a cyclic molecule containing this linkage between a serine and a threonine residue, which could serve as a model for an *intramolecular* phosphodiester linkage in a protein. Drummond[9] showed that, on the basis of the distance between α-carbons of all possible serine–threonine pairs, two *intramolecular* phosphodiester linkages are possible in *Azotobacter* flavodoxin, giving rise to a nine- and a 15-membered ring, respectively.[10] Recently, Van Boom et al.[11] synthesized a molecule, consisting of an isolated serine and threonine connected via a phosphodiester linkage, which could serve as a model for an *intermolecular* phosphodiester linkage.

Another reason for synthesizing a cyclic molecule containing a phosphodiester linkage was that macrocycles containing a phosphate moiety are not widely studied.[12] The presence of P=O moieties in macrocycles could lead to interesting molecular hosts capable of binding, e.g., metal ions and organic bases.[12,13]

Finally, since a phosphodiester linkage may actually be capable of stabilizing the peptide structure (vide supra), in particular, a secondary structure element, we plan to apply this linkage for the stabilization of a turn[10] and helix.

Here we describe the first synthesis of a cyclic phosphopeptide **5** and summarize its NMR spectroscopic features.

For the synthesis of a cyclic phosphodiester containing peptide we chose the sequence Thr-Gly-Ser. We considered a tripeptide a more favorable system than the dipeptide Thr-Ser, since the latter will give rise to only a nine-membered ring containing a trans amide bond. For the middle amino acid we chose Gly, because the allowed ϕ,ψ conformational space of this amino acid is larger than for an amino acid with a side chain.[14] In addition, this amino acid will simplify NMR spectra, which is desirable for a conformation analysis.[15]

The synthesis of the precursor for the macrocyclization was straightforward and is outlined in Scheme I. Starting from commercially available Boc-Ser(OBzl)-OH (**1**), the macrocyclization precursor **2** is obtained in 54% overall yield. The phosphitylating agent **3** was prepared from PCl_3 in two steps analogous to the procedure described for bis(*N*,*N*-diisopropylamino) benzyl phosphoramidite.[16]

Slow addition of a solution of the phosphitylating agent[17] **3** to a solution[18] containing the tripeptide **2** and 2 equiv of 1*H*-tetrazole afforded, after oxidation with *tert*-butyl hydroperoxide and subsequent purification by gel filtration (Sephadex LH-20) and short-column chromatography over silica, the cyclic phosphotriester peptide **4**. Because the obtained product almost coeluted with the starting material **2** from the Sephadex column, we suspected that we had in fact obtained the triester **4** and not a symmetrical dimer, which would have eluted significantly earlier. This expectation was confirmed by the FAB-mass spectrum of **4**.[19] ^{1}H, ^{13}C, and ^{31}P NMR spectra were in excellent agreement with the proposed structure. The diastereomeric phosphotriesters, which

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(10) The synthesis of these cyclic phosphopeptides is the progress and will be reported soon. If present at the particular location in *Azotobacter* flavodoxin, the phosphodiester linkage may actually stabilize the turn comprising a serine(157)–threonine(160) pair.[9]
(11) Dreef-Tromp, C. M.; Erkelens, C.; Van Der Marel, G. A.; Van Boom, J. H. *J. Am. Chem. Soc.* **1989**, *111*, 6470.
(12) For a recent review on phosphorus-containing macrocycles: Tsvetkov, E. N.; Bovin, A. N.; Syundyukova, V. Kh. *Usp. Khim.* **1988**, *57*, 1353.
(13) The P=O moiety in, e.g., triphenylphosphine is a good proton acceptor and can form complexes with a variety of organic bases; see, e.g.: Etter, M. C.; Baures, P. W. *J. Am. Chem. Soc.* **1988**, *110*, 639 and references cited therein.
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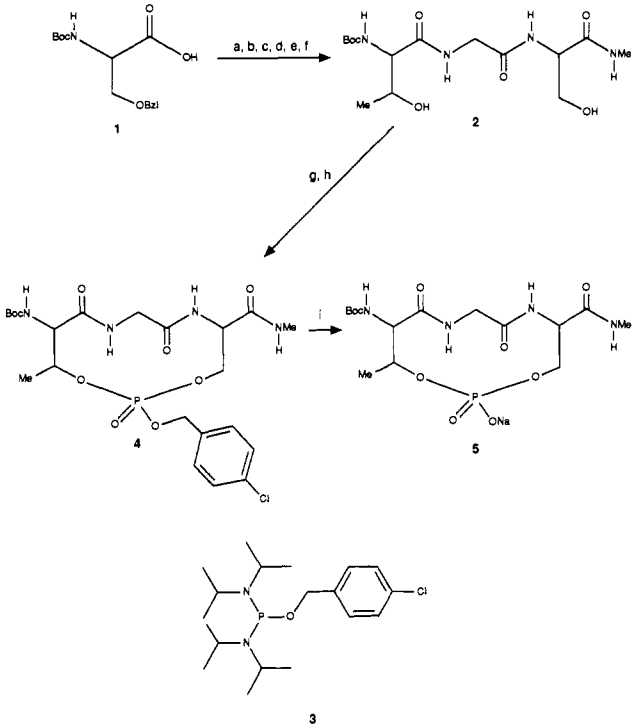
(15) This work will be published in due course.
(16) Dreef, C. E.; Elie, C. J. J.; Hoogerhout, P.; Van Der Marel, G. A.; Van Boom, J. H. *Tetrahedron Lett.* **1988**, *29*, 6513. In view of the lability of this phosphitylating agent, it is advisable to prepare the bis(*N*,*N*-diisopropylamino) 4-chlorobenzyl phosphoramidite when needed. This reagent was also prepared by Caruthers et al. (Caruthers, M. H.; Kierzek, R.; Tang, J. Y. In. *Biophosphates and Their Analogues—Synthesis, Structure, Metabolism and Activity*; Bruzik, K. S., Stec, W. J., Eds.; Elsevier Science Publishers: Amsterdam, 1987; p 3) in a different manner.
(17) The phosphitylating agent **3** (2 equiv) was added over a time period of 30 h. Excess of the reagent is necessary because of decomposition during addition. *Slow* addition, however, was thought necessary to avoid diphosphitylation.
(18) The starting concentration of tripeptide **2** was 50 mM; more diluted solutions to avoid *intermolecular* reactions did not improve the yield. Surprisingly, dimeric products could not be detected in the reaction mixture at this relatively high concentration.
(19) The FAB-mass spectrum showed major peaks at m/z 585 $(M + Na)^+$ and m/z 563 $(M + H)^+$. We thank Dr. R. Fokkens of the Institute for Mass Spectroscopy of the University of Amsterdam for recording the FAB-mass spectra.

Scheme I[a]



[a] (a) Isobutyl chloroformate, 4-ethylmorpholine, H_2NMe (82%). (b) TFA, CH_2Cl_2, Et_3N. (c) Isobutyl chloroformate, 4-ethylmorpholine, Boc-Gly-OH (step b + step c, 82%). (d) TFA, CH_2Cl_2. (e) DCC, HOBt, Boc-Thr(OBzl)-OH, 4-ethylmorpholine, (step d + step e, 89%). (f) Pd/C, MeOH, H_2 (90%). (g) **3** (2 equiv), 1*H*-tetrazole, MeCN. (h) *t*-BuOOH (step g + step h, 30%). (i) Pd/C, *t*-BuOH, H_2O, NaOAc (1.2 equiv), H_2, Sephadex LH-20 (92%).

could be separated by short-column chromatography, were formed in a ratio of 15:1 in 32% total yield.[20] Hydrogenolysis of the 4-chlorobenzyl group under buffered[21] conditions afforded the sodium salt of the cyclic phosphopeptide **5** in 92% yield.

The NMR data of **5** are shown in Table I. The ^1H NMR spectra of **5** and **4**[20] display very sharp signals, indicative of conformers engaged in a fast equilibrium or perhaps even the presence of a single conformer in this solvent. Comparison of the ^1H NMR data of **5** with those obtained by Live and Edmondson[7] for the serine–threonine phosphodiester linkage in *Azotobacter* flavodoxin shows that the α-CH of threonine in **5** has a chemical shift (4.18 ppm) similar to that of the corresponding proton in *Azotobacter* flavodoxin (4.0 ppm). Van Boom et al.[11] found that in serylthreonyl phosphate the α-CH of threonine resonates at 3.70 ppm. However, a striking difference in chemical shift values, as compared to those obtained by Live and Edmondson,[7] was observed for the β-C protons of the serine residue in **5**: 4.07 and 4.30 ppm, as opposed to 3.4 and 3.7 ppm in *Azotobacter* flavodoxin. These chemical shift values found for **5** are in agreement with those earlier obtained[11] for serylthreonyl phosphate: 4.14 and 4.22 ppm.

In conclusion, the described method for the introduction of a phosphodiester linkage leading to a cyclic phosphopeptide provides a versatile approach for the synthesis of other cyclic phosphopeptides. Conformational analysis[15] of these types of molecules may reveal how the phosphodiester linkage affects the structure of the macrocycle.

In addition, via this approach, other phosphate-containing nonpeptide macrocycles[12] can be synthesized possibly possessing interesting structural features and binding properties.[12,13]

Acknowledgment. We thank A. W. M. Lefeber for recording some of the NMR spectra. We thank one of the referees for drawing our attention to ref 9.

Registry No. 1, 23680-31-1; **2**, 123639-57-6; **3**, 109915-25-5; **4** (diastereomer 1), 123621-78-3; **4** (diastereomer 2), 123673-04-1; **5**, 123621-79-4; BOC-Ser(BZl)-NHMe, 90013-41-5; BOC-Gly-OH, 4530-20-5; BOC-Gly-Ser(Bzl)-NHMe, 123621-80-7; BOC-Thr(Bzl)-OH, 15260-10-3; BOC-Thr(Bzl)-Gly-Ser(Bzl)-NHMe, 123621-81-8.

(20) A satisfactory explanation for the observed diastereoselectivity cannot be offered at this time. In addition, it is not clear which diastereomer of the phosphotriester **4** is formed. The NMR data of the diastereomer **4** formed in excess: ^1H NMR (CD_3OD) δ 1.35 (d, Thr-C$^4H_3$, $J_{HH,vic}$ = 6.4 Hz), 1.52 (s, Boc), 2.78 (s, N(H)CH_3), 3.80 (d, Gly-C^2H_a, $J_{HH,gem}$ = 16.6 Hz), 4.04 (d, Gly-C^2H_b), 4.28 (ddd, Ser-C^3H_a, $J_{HH,vic}$ = 2.8 Hz, $J_{HH,vic}$ = 11.2 Hz, J_{PH} = 10.3 Hz), 4.42 (ddd, Ser-C^3H_b, $J_{HH,vic}$ = 2.8 Hz, J_{PH} = 10.8 Hz), 4.42 (t, Thr-C^2H, $J_{HH,vic}$ = J_{PH} = 2.1 Hz), 4.73 (q, Ser-C^2H, J_{PH} = 2.8 Hz), 4.96 (d, 4-Cl-benzyl, PhCH_2, J_{PH} = 8.8 Hz), 5.32 (dq, Thr-C^3H, J_{PH} = 6.4 Hz), 7.28 (s, 4-Cl-benzyl, aromatic part); ^{13}C NMR (CD_3OD) 18.0 (Thr-C^4), 26.6 (N(H)CH_3), 44.7 (Gly-C^2), 53.3 (Ser-C^2, J_{PC} = 4.4 Hz), 60.1 (Thr-C^2, J_{PC} = 10.3 Hz), 70.3 (Ser-C^3, J_{PC} = 5.9 Hz), 79.7 (Thr-C^3, J_{PC} = 4.4 Hz), 171.7, 172.6, and 174.4 (Ser-C^1, Gly-C^1, and Thr-C^1), 28.6, 81.4, and 157.7 (Boc), 69.9 (4-Cl-benzyl, PhCH_2, J_{PC} = 5.9 Hz), 129.7, 130.8, 135.5, 135.7 (4-Cl-benzyl, aromatic part); ^{31}P NMR −0.15.

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Reaction Volumes of Excited-State Processes: Formation and Complexation of the $Pt_2(P_2O_5H_2)_4^{4-}$ Excited State

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Reaction and activation volumes are usually determined for thermal processes by measuring the effect of pressure on chemical equilibria or rates.[1,2] Interpretation of this volume information often provides valuable structural and mechanistic insight into ground-state processes. However, this methodology is much less amenable to the study of excited-state processes, where generally only activation volumes can be obtained.[3,4] Partial molar volumes of short-lived species are not readily determined by using conventional methodologies. Consequently, our understanding of excited state processes is limited, in part, by our inability to construct reaction volume profiles involving excited states. In this regard, we wish to report the use of photoacoustic calorimetry (PAC) to measure the reaction volume and enthalpy for the formation and complexation of the excited state of $Pt_2(P_2O_5H_2)_4^{4-}$. This information can be used to estimate partial molar volumes and structural changes of the excited state.

Photoacoustic calorimetry measures the volume changes of a chemical system following photoexcitation.[5] The amplitude and time evolution of these changes are determined by deconvolution of the experimental acoustic waveforms. Details of this method have been previously reported.[6,7] These volume changes result

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