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MILD BASIC AND HIGHLY SELECTIVE HYDROLYSIS OF AN ARYL-ALKYL 1-H-PHOSPHONATE DIESTER: PREPARATION OF THE MONO-1-H-PHOSPHONYLATED DIPEPTIDE Z-Ser(OPO_2H_2)-Tyr(OH)NH_2.

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ABSTRACT
Basic hydrolysis (pyridine-water) of a 2,2,2-trifluoroethyl tyrosinyl 1-H-phosphonate diester affords predominantly a 2,2,2-trifluoroethyl 1-H-phosphonate mono-ester and tyrosine. The latter finding has been applied to the synthesis of a dipeptide consisting of a 1-H-phosphonylated serine and a non-1-H-phosphonylated tyrosine moiety.

In an earlier paper[1] we showed that 1-H-phosphonate monoesters of serine (1) and tyrosine (3) were easily accessible by phosphitylating of the corresponding protected amino acids with the monofunctional reagents 5 or 6. It was also demonstrated that 1 and 3 could be coupled, in the presence of pivaloyl chloride (PVCl), with an appropriately protected nucleoside to give, after oxidation of the intermediate 1-H-phosphonate diesters, the nucleopeptide fragments 2 and 4, respectively.

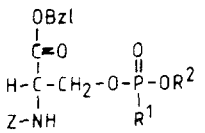
We now report that mixed 1-H-phosphonate diesters 7 can be hydrolyzed, with a high degree of selectivity, under mild basic conditions. The latter will be illustrated in the preparation of the mono-1-H-phosphonate dipeptide Z-Ser(OPO_2H_2)-Tyr(OH)NH_2 (13).

The use of 1-H-phosphonate diesters as intermediates in the synthesis of nucleic acids is not as well advanced as the corresponding phospho[2]- or phosphite[3]-triester approaches. The reason for this is mainly due to the inherent instability of 1-H-phosphonate diesters towards base. Hata et al.[4] circumvented this problem by converting the P-H bond, present in a 1-H-phosphonate diester, into a relatively more base-stabile P-acyl(4-chlorobenzoyl) bond.

The recent and interesting finding that DNA[5] and RNA[6] could also be synthesized by a solid-phase approach, in which intermediate 1-H-phosphonate diester linkages play an essential role, stimulated us to study in detail the stability of these type of bonds towards base.

Apart from some earlier studies on this subject[7], Gibbs et al.[8] reported that ammonolysis of the 1-H-phosphonate diesters 7 (R=Alkyl or Aryl) containing a trifluoroethyl group afforded predominantly, according to pathway A, the 1-H-phosphonate monoesters 8. On the possible formation of 9 (pathway B) starting from the mixed aryl-diester 7 (R=Aryl) was not commented upon. However, we assumed that basic hydrolysis of 7 (R-Aryl) would follow the same pattern as established[9] for an aryl internucleosidic phosphotriester bond: i.e., the rate and selective formation of an internucleosidic phosphodiester linkage is determined by the pK of the leaving aryloxy group and the nature of the base, respectively.

In order to verify, and eventually extend the findings of Gibbs et al. toward the preparation of phosphorylated peptides, we performed the following experiments. The crude serine H-phosphonate ester 7a was prepared[1] by adding PVCl (1.1 mmol) to a solution of 1 (1 mmol) and 2,2,2-trifluoroethanol (1.2 mmol) in pyridine (2.5 ml) and acetonitrile (2.5 ml). Monitoring of the coupling by ^{31}P-NMR revealed complete conversion of 1 into 7a (δP 10.12 and 9.88 ppm; JP-H 737.3 Hz) within 10 min. Following the same protocol a solution of 7b (δP 6.16; JP-H



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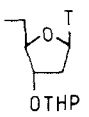
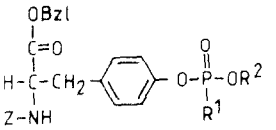
2 $R^1 = OH$; R^2 = 



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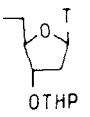
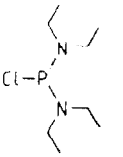
4 $R^1 = OH$; R^2 = 



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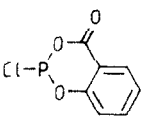

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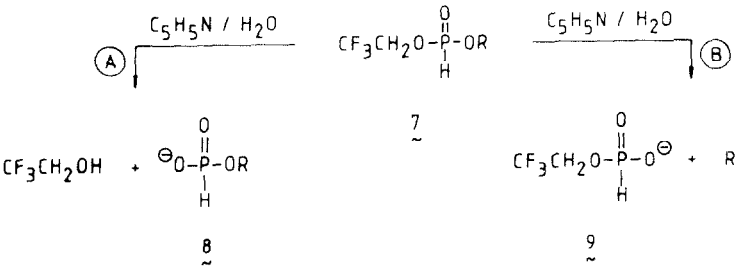
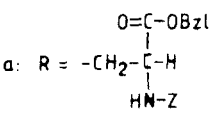
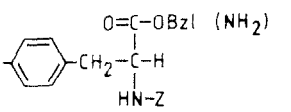
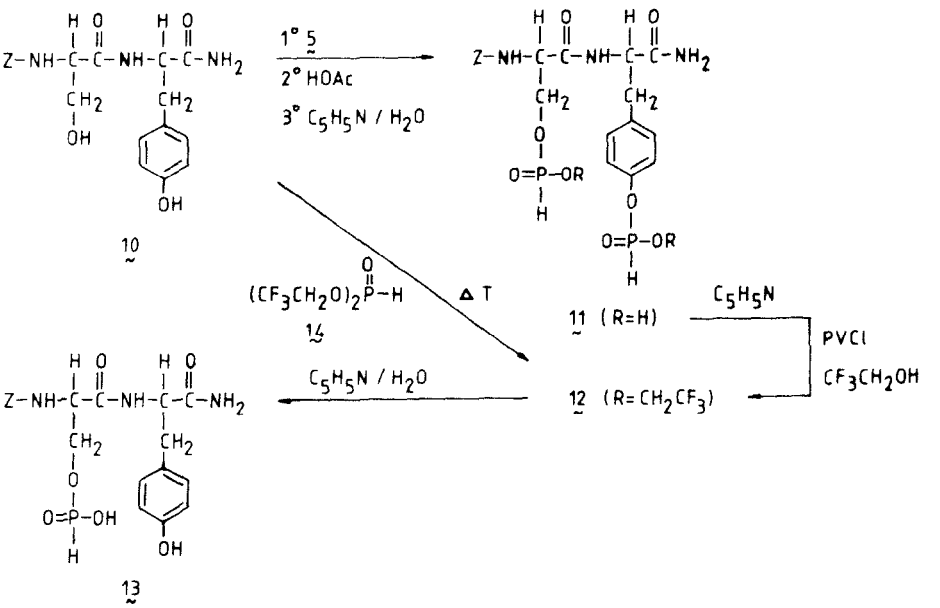


7 8 9

a: R = 

b: R = 



10 11 (R=H) 12 (R=CH_2CF_3) 13 14

744.2 Hz) was obtained. Hydrolysis of 7a and 7b was now effected by adding water (0.5 ml) to the individual solutions. Work-up, after 10 min., and purification of the mixture afforded, in the case of 7a, homogeneous serine H-phosphonate 8a (δP 5.2 ppm; JP-H 632.3 Hz) in a yield of 85%. On the other hand, work-up and purification of the hydrolysis products of 7b gave crystalline Z-Tyr(OH)OBzl (m.p. 113-115°C; $[\alpha]_D^{20}$ (c=1, MeOH) -11.9°) in 75% yield. The above results indicate that the hydrolysis of 7a (di-alkyl ester) proceeds as expected[8] via path A. The outcome of the hydrolysis of the aryl-alkyl diester 7b is, however, in contrast with the finding[8] that ammonolysis of 7 (R=phenyl) gave 8 (R=phenyl) in a yield of 80%. Two possible explanations can be forwarded to explain the observed ambiguity in the basic hydrolysis of the alkyl-aryl diester 7b: (i) the nature of the base determines the pathway (A or B) to be followed; (ii) formation (via path A) of 8b which is then further hydrolyzed to give Z-Tyr(OH)OBzl and phosphorous acid. Both possibilities proved to be not viable. Thus ammonolysis of 7b (R=Tyrosine amide) afforded Z-Tyr(OH)NH_2 in a high yield. Further, 8b was rather stable in pyridine-water: no detectable degradation was observed (TLC-analysis) after 1 h at 20°C. However, saponification of 8b (R=Tyrosine amide) with sodium hydroxide (0.1N) resulted in a rapid formation (TLC-analysis) of Z-Tyr(OH)NH_2[11].

An interesting application of the hydrolysis via pathway B of an aryl-alkyl H-phosphonate diester will be demonstrated in the preparation of the dipeptide Z-Ser(OPO_2H_2)-Tyr(OH)NH_2 (13).

Dipeptide 10[12] was phosphitylated with 5 followed by acidolysis and subsequently hydrolysis with pyridine-water, to afford, after purification, the bis-1-H-phosphonylated derivative 11[10] (R=H; Rf 0.24 (CH_3CN/H_2O, 9:1); δP 5.8 (JP-H 629.9) and 2.0 (JP-H 634.8 Hz)) in a yield of 50%. Coupling of 11 with trifluoroethanol in pyridine, and in the presence of PVCl, gave intermediate 12 (δP 9.92 (JP-H 737.2 Hz) and 6.32 (JP-H 744.6 Hz)) which was then hydrolyzed. Work-up and purification (DEAE-Sephadex A-25) yielded homogeneous[13] 13[10] (yield 70%, Rf 0.46 (CH_3OH/H_2O, 9:1), δP 5.20 (JP-H 625.0 Hz)). The mono-H-phosphonylated dipeptide 13 could also be prepared (overall yield 65%, based on 10) by hydrolyzing intermediate 12 obtained by transesterification of 10 with 14[8].

In conclusion the results presented in this paper may open the way of preparing oligopeptides containing solely 1-H-phosphonylated serine and/or threonine moieties. It is also not excluded that the 1-H-phosphonate functions may, in turn, give an easy access[14] to modified or non-modified phosphate derivatives of biologically important oligopeptides[15].

ACKNOWLEDGEMENT

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REFERENCES AND NOTES

1. E. Kuyl-Yeheskiely, C.M. Tromp, A.H. Schaeffer, G.A. van der Marel and J.H. van Boom, Nucl. Acids. Res., 15 , 1807 (1987).
2. C.B. Reese, Tetrahedron, 34, 3143 (1978).
3. E. Sonveaux, Bioorg. Chem., 14, 274 (1986).
4. A. Kume, M. Iujii, M. Sekine and T. Hata, J. Org. Chem., 49, 2139 (1984).
5. B.C. Froehler, P.G.Ng and M.D. Matteucci, Nucl. Acids Res., 14, 5399 (1986). P.J. Garegg, I. Lindh, T. Regberg, J. Stawinski and R. Strömberg, Tet. Lett., 27, 4051 (1986).
6. P.J. Garegg, I. Lindh, T. Regberg, J. Stawinski and R. Strömberg, Tet.Lett., 27, 4405 (1986)

7. P.R. Hammond, J. Chem. Soc., 2521 (1962). E.N. Walsh, J. Am. Chem. Soc., 81; 3023 (1959).
8. D.E. Gibbs and C. Larsen, Synthesis, 410 (1984).
9. C.B. Reese and L. Zard, Nucl. Acids Res., 9, 4611 (1981).
10. ^{1}H-NMR (200 MHz) data (δ-values in ppm) of compounds 10 and 13. 10 ($CDCl_3/CD_3OD$): 7.40-7.35, 5H (Arom.); 7.06-7.02, d, 2H (Φ Tyr) J 8.5 Hz; 6.77-6.72, d 2H (Φ Tyr), J 8.28 Hz; 5.09-5.07, d, 2H (CH_2 Z), J 2.44 Hz; 4.6-3.6, 4H (2x α-CH, β-CH_2 Ser); 3.10-2.85, 2x d, 2H (β-CH_2 Tyr). 13 ($CDCl_3/CD_3OD/D_2O$): 8.54 and 5.38, 2x ½H, J 630.3 Hz; 7.59, bs, 5H (Arom.); 7.35-7.30, d, 2H (Φ Tyr), J 8.04 Hz; 7.01-6.97, d, 2H (Φ Tyr), J 8.04 Hz; 5.32, s, 2H (CH_2 Z); 4.77-4.24, 4H (2x α-CH, β-CH_2 Ser); 3.31-2.77, 2H (CH_2 Tyr). ^{13}C-NMR (200 MHz) data of compounds 10, 11 and 13. 10 ($CDCl_3/CD_3OD$/DMSO): 173.0 and 169.6, 2x CO (Ser/Tyr); 154.8 CO (Z); 129.2-114.2 (C-Arom.); 65.6 and 61.2 (2x CH_2 Z and β-CH_2 Ser); 55.9 and 53.4 (2x αCH/Ser/Tyr); 35.4 (β-CH_2 Tyr). 11 ($CDCl_3/CD_3OD$): 175.0 and 171.3 (2x CO/Tyr/Ser); 151.7-121.3 (C-Arom.); 67.7 and 63.8 (2x CH_2 Z and β-CH_2-Ser); 57.1 and 55.7 (2x α-CH); 37.5 (β-CH_2 Tyr). 13 ($CDCl_3/CD_3OD$): 173.7 and 169.6 (2x CO/Ser/Tyr); 155.1 CO (Z); 131.7-114.4 (C-Arom.); 66.1 (CH_2 Z); 62.0 (β-CH_2 Ser); 55.2 and 53.9 (2x αCH/Ser/Tyr); 35.7 (β-CH_2-Tyr).
11. The second possibility (ii) was also excluded by the fact that hydrolysis (pyridine/water) of 7b afforded, in agreement with pathway B, Z-Tyr(OH)OBzl and 9. The latter compound was in every aspect (^{31}P- and ^{1}H-NMR) identical with an authentic[8] sample of 9.
12. The dipeptide 10 (m.p. 184-186°C, Rf 0.65 ($MeOH/CH_2Cl_2$, 15:85)) was prepared (yield 85%) by coupling, mixed anhydride method (J. Am. Chem. Soc., 94, 6190, 1972), Z-Ser-OH with Tyr-NH_2 (see reference 10 for NMR data).
13. Anal. Calc. for $C_{20}H_{23}N_3O_8PNa$: P 6.36; Found: P 6.30.
14. For example the conversion of a 1-H-phosphonate mono-ester into the corresponding phosphorothioate or phosphate esters has been described by Hata et al., Tet. Lett., 3943 (1974).
15. P.A. Bartlett and L.A. Lamden, Bioorg. Chem., 14, 356 (1986). D. Kick, P. Barret, A. Cummings and J. Sommerville, Nucl. Acids Res., 15, 4099 (1987). H. Mori, J. Kondo and Y. Ihara, Science, 235, 1641 (1987). W. Bannwarth and A. Trzeciak, Helv. Chim. Acta, 76, 175 (1987).

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