80. A New Reagent for Polypeptide Synthesis: μ-Oxo-bis-[tris-(dimethylamino)-phosphonium]-bis-tetrafluoroborate

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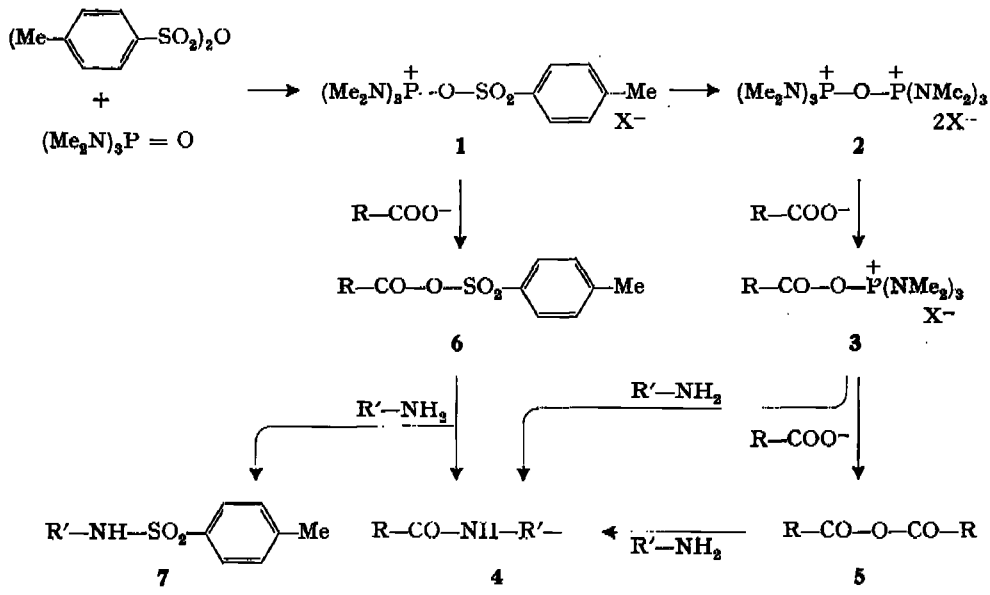
Summary. The products **1** and **2** (X = OTs) have been isolated from the reaction between hexamethylphosphortriamide and *p*-toluene sulfonic anhydride and the latter converted into μ-oxo-bis-[tris-(dimethylamino)-phosphonium]-bis-tetrafluoroborate **2** (X = BF_4). This is a practical reagent for the formation of the peptide link. Where racemisation is possible via oxazolone formation this can be decreased by the addition of 1-hydroxybenzotriazole or N-hydroxysuccinimide. These additives may also increase the efficiency of condensation at glycine and proline residues.

Some years ago, two of us published data, together with another author [1], purporting to substantiate *Scheme 1* (**1** → **2** → **3** → **4**) for peptide synthesis. In subsequent studies there emerged serious defects in the experimental work on which

Scheme 1



these conclusions were based [2]. The first disquieting feature was the amount of N-*p*-toluene sulfonyl derivative **7** of the amine $R'NH_2$ isolated as a by-product using this method. This could readily be explained by incomplete formation of **2** (X = OTs)

resulting in reaction of $RCOO^-$ with **1** (X = OTs) to give the unsymmetrical anhydride **6** which could afford both the desired amide **4** and the N-*p*-toluene sulfonyl derivative **7** by nucleophilic attack of R'–NH_2. Secondly, the claims concerning low racemisation during coupling could not be substantiated.

In a complete reinvestigation of the matter [3] we have indeed isolated the postulated intermediates **1** (X = OTs) and **2** (X = OTs), although the reaction conditions had to be more vigorous than those reported earlier. Moreover, we wish to report the preparation of a new crystalline reagent[1]), μ-oxo-bis-[tris-(dimethylamino)-phosphonium]-bis-tetrafluoroborate **2** (X = BF_4).

Addition of *p*-toluene sulfonic anhydride to hexamethylphosphortriamide (HMPT) at 20° afforded a new crystalline precipitate within 5–20 minutes under anhydrous conditions. This proved to be salt **1** (X = OTs), m.p. 76–78°, which is exceedingly sensitive to moisture. Because of this, **1** (X = OTs) is not normally isolated but rather converted *in situ* by further reaction with HMPT at 55° for 3 h into the desired intermediate **2** (X = OTs), m.p. 115–117°. During this latter process dissolution of the first precipitate of **1** (X = OTs) was followed by crystallisation of **2** (X = OTs). In order to circumvent the problems associated with the hygroscopic nature of **1** (X = OTs) and **2** (X = OTs), the anion was exchanged for BF_4^- by reaction of these salts with $NaBF_4$ in acetonitrile, affording *p*-toluene sulfonyl-[tris(dimethylamino)-phosphonium]-tetrafluoroborate **1** (X = BF_4), m.p. 115–120° and μ-oxo-bis-[tris-(dimethylamino)-phosphonium]-bis-tetrafluoroborate **2** (X = BF_4), m.p. 194–204°, respectively having satisfactory analytical and NMR. data (Table 1). The double decomposition leading to **1** (X = BF_4) and **2** (X = BF_4) was possible because of the insolubility of sodium *p*-toluenesulfonate relative to $NaBF_4$ in acetonitrile.

Table 1. *NMR. data of HMPT derivatives*

	^{31}P-NMR. (^{1}H-noise-decoupled) [a])	^{1}H-NMR. [b])
$(Me_2N)_3P=O$	–24.7	2.57 (*Me*-N, d, J = 9 Hz)
$(Me_2N)_3\overset{+}{P}$-O-SO_2-  -Me	–33.6	2.77 (*Me*-N, 18 H, d, J = 11 Hz)
BF_4^-		2.42 (*Me*-Ar, 3 H, s) 7.70 (*H*-Ar, 4 H)
$(Me_2N)_3\overset{+}{P}$-O-$\overset{+}{P}(NMe_2)_3$ $2BF_4^-$	–29.6	2.83 (*Me*-N, J = 11 Hz) [d])
$(Me_2N)_3\overset{+}{P}$-O-P(O)$(NMe_2)_2$ [c])	–27.0	2.53 (*Me*-N, 12H, d, J = 10.7 Hz)
Ph_4B^-	–10.2	2.37 (*Me*-N, 18H, d, J = 10.8 Hz)

[a]) Spectra measured in CH_3CN/CD_3CN, signals in ppm relative to H_3PO_4 external reference.
[b]) CD_3CN solution, signals in ppm relative to Me_4Si standard.
[c]) Data from *Macchia et al.* [4] using $CDCl_3$ solutions.
[d]) Due to P-----P coupling the Me splitting becomes characteristic of an $X_3AA'X'_3$ system [13] and is thus not a simple doublet.

[1]) Marketed by *Fluka* AG. as *Bates'* Reagent (11595).

Bates' reagent **2** (X = BF_4) is a white crystalline solid which is insoluble in Et_2O, EtOAc, THF, CH_2Cl_2, and $CHCl_3$ but soluble in MeCN, DMF and somewhat less soluble in HMPT. Although the reagent reacts with water it does not appear to be very hygroscopic, and it can be manipulated without the precautions required for the corresponding di-*p*-toluenesulfonate **2** (X = OTs). The reagent **2** (X = BF_4) has been used in stepwise peptide synthesis in MeCN, DMF or HMPT as solvent at room temperature, using **2** (X = BF_4), N-methylmorpholine (NMM), Z, BOC- or Bpoc-amino acid and amino component in the molar ratios 1.5:2:1:1. The protected pentapeptides **8** and **9** were conveniently synthesised in this way.

Bpoc–Ser(Bu^t)–Thr(Bu^t)–Asp(OBu^t)–Tyr(Bu^t)–Gly–OPh

8

Z–Ala–Leu–Nva–Ser(Bu^t)–Gly–OPh

9

According to *Scheme 1* the activated carboxylic acid derivative involved in amide formation could be either the acyloxyphosphonium salt **3** (X = BF_4) or the symmetrical anhydride **5**. However, in spite of considerable efforts using ^{31}P-NMR., no evidence could be obtained for the intermediacy of **3** (X = BF_4) in the nucleophilic attack by R'–NH_2. A low temperature ^{31}P-NMR. study of the reaction of *Bates'* reagent **2** (X = BF_4) with Z–Gly–OH in the presence of NMM in DMF showed no apparent reaction at −40°, but at +3° HMPT was liberated over 1.5 h. There was no evidence for another phosphorus-containing species such as **3** (X = BF_4). In order to probe this more deeply, [1-^{13}C] and [2-^{13}C]-Z–Gly–OH were used to give the added dimension of ^{13}C–^{31}P spin-spin coupling in the search for activated carboxyl intermediates. After 11 minutes signals were observed which could be assigned to the symmetrical anhydride **10**[2]. This was checked by comparison with authentic material prepared from the appropriately labelled Z–Gly–OH and DCCI [5]. After 48 hours the symmetrical anhydride **10** was found to have rearranged partly to N,N'-bis(benzyloxycarbonyl)-glycylglycine **11**, which was identified by comparison with authentic material produced on refluxing **10** in benzene [6].

$(Z–NH–CH_2–CO)_2O$ **10**

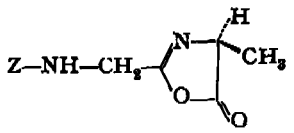
Z–NH–CH_2–CO–N(Z)–CH_2–COOH **11**

Thus, although acyloxyphosphonium salts of the type **3** have been postulated as active intermediates in amide formation using a variety of phosphorus compounds [7] [8], there is no conclusive evidence to support this. It must be admitted, however, that anhydride formation using *Bates'* reagent **2** (X = BF_4) *in the absence* of the nucleophile R'–NH_2 does not exclude participation of the fugitive acyloxyphosphonium salt **3** (X = BF_4) in the presence of R'–NH_2, which is a better nucleophile than R–COO^-.

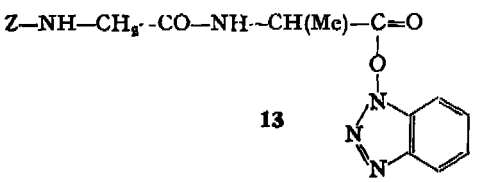
When *Bates'* reagent was employed in the *Izumiya* test [9] (Z–Gly–Ala–OH → Z–Gly–Ala–Leu–OBzl) it was found that the degree of racemisation varied according to the strength of the base used [Et_3N (43%), NMM (16%), poly *Hünig* base (9%)]. This strongly suggested the intermediacy of the oxazolone **12** as being responsible

[2] *Castro & Dormoy* (7) have also prepared anhydrides by analogous use of phosphonium salts.

for racemisation. Treatment of Z–Gly–Ala–OH in MeCN with *Bates'* reagent followed by 0.5 equivalents of Amberlyst A21, a weakly basic macroreticular anion exchange resin, gave IR. evidence (1832 cm^{-1}) for **12**. In DCCI coupling reactions this source of racemisation has been drastically reduced by the addition of N-hydroxysuccinimide (HONSu) [10] or 1-hydroxybenzotriazole (HOBt) [11], which rapidly form activated esters of the carboxyl component not prone to racemisation or side reactions to which oxazolones are susceptible. Addition of HOBt to the *Izumiya* test involving *Bates'* reagent brought about a marked reduction in racemisation [NMM (3%), poly *Hünig* base (1%)] due to the participation of **13**.



12



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In our current programme involving the synthesis of an analogue of lysozyme we have tested *Bates'* reagent + HONSu for fragment couplings where racemisation is not a problem, in order to minimise deleterious side reactions occasioned by the active 4-position of oxazolone intermediates. The method has been used for coupling of fragments **14** + **15**, **16** + **17**, and **18** + **19**, indicated[3]), and the results are very favourable in comparison with established methods, particularly with respect to ease of purification of the products. The problem of removal of dicyclohexylurea from insoluble protected peptides is obviated by using *Bates'* reagent. In the case of the octapeptide Bpoc(**14** + **15**)OPh the DCCI + HOBt product required extensive purification, whereas *Bates'* reagent alone afforded a product which could be isolated by crystallisation.

Bpoc–Orn(Adoc)–Thr(Bu^t)–Pro–Gly–OH **14** H–Ser(Bu^t)–Ala–Asn–Gly–OPh **15**

Bpoc–Cys(Acm)–Asn–Ile–Pro–OH **16** HCys(Acm)–Ala–Ala–Leu–Nva–Ser(Bu^t)–Gly–OPh **17**

Bpoc–Cys(Acm)–Asn–Ile–Pro–Cys(Acm)–Ala–Ala–Leu–Nva–Ser(Bu^t)–Gly–OH **18**

H–Asp(OBu^t)–Ile–Thr(Bu^t)–Ala–Ser(Bu^t)–Val–Gly–OPh **19**

Experimental Part

M.p. were determined using a *Kofler* Block except for salts **1** and **2**, which were examined in sealed capillaries. IR. spectra were recorded on a *Pye-Unicam* SP. 200. NMR. spectra were obtained using *Varian* Associates HA. 100 or XL. 100 instruments. Optical rotation measurements were made on a *Thorn Bendix* ETL-NPL-143A automatic polarimeter. Amino acid analyses were carried out by a *Jeol* JLC-5AH automatic analyser with digital integrator unit.

[3]) The fragments used were synthesised by standard methods and have satisfactory analytical data. A full description of this work will be published separately.

Gel filtration was carried out using Sephadex LH20 swollen in distilled DMF and monitored by LKB *Uvicord* UV (280 nm) and *Thorn Bendix* Automatic Polarimeter 143D at 546 nm using a 2 mm × 28 mm flow cell.

Thin layer chromatograms (TLC.) were run on silica gel (*Merck* Kieselguhr GF 254) usually with the addition of starch (10% by weight). Visualisation of the product spots was achieved by one of the following methods: (i) UV. light (254 nm); (ii) ninhydrin 0.5% in butanol spray followed by heat; (iii) fluorescamine 1% in acetone spray followed by UV. light (360 nm); (iv) I_2 vapour; (v) Cl_2/ClO_2 followed by 1% *w/v* aqueous KI.

The following solvent systems served as eluants:
A) $CHCl_3$/MeOH 19:1; B) $CHCl_3$/MeOH 9:1; C) $CHCl_3$/MeOH 3:1; D) $CHCl_3$/Pr^iOH 9:1; H) BuOH/HOAc-H_2O 4:1:5 upper phase; M) MeCN/H_2O 9:1; N) $MeCO_2Et$/benzene 1:1; R) $MeCO_2Et$/benzene 2:1.

1. p-*Toluenesulfonyl-[tris-(dimethylamino-)phosphonium]tetrafluoroborate* (**1**, X = BF_4). *p*-Toluene sulfonic acid anhydride[4]) (7.8 g, 24 mmol) was dissolved in HMPT (24 ml) and stirred at room temperature. After about 5 min the reaction mixture became cloudy and the *p*-toluene sulfonate **1** (X = OTs) precipitated slowly. After a further 15 min the solid was filtered off (dry box, N_2 atmosphere) and washed with ether to give *p*-toluenesulfonyl[tris-(dimethylamino-)phosphonium]*p*-toluenesulfonate (**1**) (X = OTs), m.p. 76–78°.

$C_{20}H_{32}N_2O_6PS_2$	Calc.	C 47.51	H 6.38	N 8.31%
(505.6)	Found	,, 47.41	,, 6.75	,, 7.95%

The mono-*p*-toluene sulfonate (4.43 g, 8.85 mmol) was dissolved in dry acetonitrile (25 ml) and $NaBF_4$ (0.962 g, 8.85 mmol) then added; the suspension was stirred vigorously for 16 h. The sodium *p*-toluene sulfonate was removed by filtration and dry ether was added to the filtrate to precipitate the monotetrafluoroborate **1** (X = BF_4) which was filtered off and washed with ether (dry box, N_2 atmosphere) to give *p*-toluene sulfonyl-[tris(dimethylamino)-phosphonium]-tetrafluoroborate (**1**) (X = BF_4), m.p. 115–120°.

$C_{19}H_{35}BF_4N_3O_3PS$	Calc.	C 37.07	H 5.98	B 2.57	F 18.04	
(421.2)		N 9.98	O 11.40	P 7.35	S 7.61%	
	Found[5])	C 36.70	H 5.83	N 9.67%		
		C 37.06	H 6.03	N 9.95	O 11.22%	(O-% by difference)
		B 2.61	F 18.01	P 7.40	S 7.72%	

2. *μ-Oxo-bis-[tris-(dimethylamino-)phosphonium]bis-tetrafluoroborate* (**2**) (X = BF_4). *p*-Toluene sulfonic anhydride (35 g, 0,11 mol) was dissolved in HMPT (150 ml) and stirred at room temperature. Precipitation of the mono-*p*-toluene sulfonate **1** (X = OTs) commenced after several minutes. The reaction mixture was stirred at room temperature for 1.5 h and then at 55° for 3 h during which time conversion to the bis-*p*-toluene sulfonate was observed. After 16 h at room temperature the reaction mixture was cooled to −30° for 3 h, then filtered (dry box, N_2 atmosphere) to give μ-oxo-bis-[tris-(dimethylamino-)phosphonium]-bis-*p*-toluene sulfonate (**2**) (X = OTs) as a hygroscopic solid (44.3 g, 60%), m.p. 115–117°.

$C_{26}H_{50}N_6O_7P_2S_2$	Calc.	C 45.60	H 7.36	N 12.27%
(684.8)	Found	,, 44.92	,, 7.50	,, 11.89%

The bis-*p*-toluene sulfonate **2** (X = OTs) (25.7 g, 36.5 mmol) in acetonitrile (125 ml) was treated with $NaBF_4$ (8.1 g, 73.0 mmol) and the suspension vigorously stirred for 16 h. After filtration of the sodium *p*-toluene sulfonate, dry ether was added to the filtrate to precipitate the product which was filtered off, washed with dry ether and dried to give μ-oxo-bis-[tris-(dimethylamino-)phosphonium]bis-tetrafluoroborate (**2**) (X = BF_4) (15.2 g, 80%), m.p. 194–204°.

$C_{12}H_{36}B_2F_8N_6OP_2$	Calc.	C 27.93	H 7.03	B 4.19	F 29.45	N 16.29	O 3.10	P 12.01%
(516.1)	Found	,, 27.73	,, 7.00	–	–	,, 15.88	–	– %
	,, [5])	,, 27.89	,, 7.20	,, 4.25	,, 29.49	,, 16.19	,, 3.06[a])	,, 11.92%

[a]) As difference.

[4]) Prepared by the method of *Field* [12] must be of good quality for this work.

[5]) Analysis carried out by *Dornis & Kolbe*, Mülheim (Ruhr).

3. *Synthesis of Bpoc-Ser(But)-Thr(But)-Asp(OBut)-Tyr(But)-Gly-OPh* (**8**). – 3.1. *Z-Tyr(But)-Gly-OPh.* Z-Tyr(But)-OH. DCHA salt (6.0 g, 11 mmol) was converted to the corresponding free acid by 20% citric acid. Extraction into ethyl acetate, drying and evaporation *in vacuo* yielded an oil (3.7 g, 10 mmol) which was dissolved in DMF (20 ml). Solutions of HBr.HGly-OPh (2.32 g, 10 mmol) and *Bates'* reagent (7.74 g, 15 mmol) each in DMF (30 ml) were added followed by NMM (3.03 g, 30 mmol). After 16 h the solvent was removed *in vacuo* at 30°. The resulting oil was partitioned between water and ethyl acetate and the organic phase washed with 5% $NaHCO_3$-solution, 10% citric acid solution, water and brine. After drying ($MgSO_4$) and evaporation of the solvent *in vacuo*, the product was purified by precipitation from ethyl acetate by petroleum ether to give Z-Tyr(But)-Gly-OPh (3.48 g, 69%), m.p. 83–86°, $[\alpha]_D^{25} = +10.2°$ ($c = 1$, $CHCl_3$); TLC. 0.6 (N), 0.7 (A)

$C_{29}H_{32}N_2O_6$ (504.6) Calc. C 69.03 H 6.39 N 5.55% Found C 68.95 H 6.46 N 5.33%

3.2. *Z-Asp(OBut)-Tyr(But)-Gly-OPh.* A solution of Z-Tyr(But)-Gly-OPh (2.83 g, 5.61 mmol) and *p*-toluene sulfonic acid monohydrate (1.07 g, 5.61 mmol) in DMF (40 ml.) was hydrogenated in the presence of Pd/C (10%) (280 mg) for 6 h, then filtered through celite.

Z-Asp(OBut)-OH (1.81 g, 5.61 mmol) and *Bates'* reagent (4.34 g, 8.4 mmol) in a minimum volume of DMF were added to the above solution followed by NMM (1.7 g, 16.8 mmol) and the reaction mixture stirred for 16 h at room temperature. After the usual work-up the product was crystallised from ethyl acetate/hexane to give Z-Asp(OBut)-Tyr(But)-Gly-OPh (3.01 g, 80%), m.p. 125–127°; $[\alpha]_D^{25} = -12.6°$ ($c = 2$, $CHCl_3$); TLC. 0.8 (B), 0.5 (A), 0.6 (N).

$C_{37}H_{45}N_3O_9$ (675.8) Calc. C 65.76 H 6.71 N 6.22% Found C 65.75 H 6.76 N 6.30%

3.3. *Z-Thr(But)-Asp(OBut)-Tyr(But)-Gly-OPh.* The fully protected tripeptide (338 mg, 0.5 mmol) described above, *p*-toluene sulfonic acid monohydrate (95 mg, 0.5 mmol) and Pd/C (10%) were hydrogenated for 5 h in DMF (5 ml). This reaction had to be monitored by TLC. as a second product was observed if the hydrogenolysis was prolonged.

Filtration of the hydrogenation mixture through celite gave a colourless solution to which was added Z-Thr(But)-OH (338 mg, 0.55 mmol) and *Bates'* reagent (4.25 mg, 0.83 mmol) then NMM (166 mg, 1.65 mmol). After 16 h stirring at room temperature followed by the usual work-up, the product was isolated and purified by crystallisation from ethyl acetate/hexane to give Z-Thr-(But)-Asp(OBut)-Tyr(But)-Gly-OPh (253 mg., 66%), m.p. 106–107°; $[\alpha]_D^{25} = -6.7°$ ($c = 1.5$, $CHCl_3$); TLC. 0.6 (B), 0.5 (A), 0.6 (N).

$C_{45}H_{61}N_4O_{12}$ (850.0) Calc. C 64.89 H 7.26 N 6.73% Found C 64.77 H 7.27 N 6.66%

3.4. *Bpoc-Ser(But)-Thr(But)-Asp(OBut)-Tyr(But)-Gly-OPh* (**8**). A solution of the above tetrapeptide (167 mg, 0.2 mmol) and *p*-toluene sulfonic acid monohydrate (38 mg, 0.2 mmol) in DMF (1 ml) was hydrogenated for 6 h over Pd/C (10%). Filtration through celite gave a colourless solution to which was added Bpoc-Ser(But)-OH (81 mg, 0.2 mmol) in DMF (0.5 ml), *Bates'* reagent (155 mg, 0.3 mmol) in DMF (1 ml) and NMM (61 mg, 0.3 mmol). After 16 h at room temperature the product was isolated in the usual way and purified by precipitation from ether by hexane. This material (154 mg) was further purified by gel filtration using LH20 with DMF as eluent to give **8** (97 mg, 49%), m.p. 108–109°, $[\alpha]_D^{25} = +8.8$ ($c = 1$, DMF), TLC. 0.6 (B), 0.6 (R).

$C_{60}H_{81}N_5O_{19} \cdot H_2O$ (1098.4) Calc. C 65.61 H 7.62 N 6.38%
Found ,, 65.64 ,, 7.56 ,, 6.68%

Amino acid analysis. Acid hydrolysis (6N HCl, 24 h, 110°): Ser 0.77, Thr 0.89, Asp 0.98, Tyr 0.91, Gly 1.00, Ser 1.04, Thr 1.03, Asp 0.98, Tyr 0.97, Gly 1.00 (corrected for destruction of Ser, Thr by extrapolation from hydrolysis at 18, 24 and 48 h).

4. *Synthesis of Z-Ala-Leu-Nva-Ser(But)-Gly-OPh* (**9**). – 4.1. *Z-Ser(But)-Gly-OPh.* Z-Ser(But)OH (1.69 g, 4.35 mmol), HBr-H-Gly-OPh (1.01 g, 4.35 mmol) and *Bates'* reagent (3.36 g, 6.5 mmol) were mixed with NMM in acetonitrile (1.43 ml, 13.0 mmol). After 13 h stirring at room temperature, the product was isolated in the usual way to give Z-Ser(But)-Gly-OPh (1.43 g, 77%). After crystallisation from ethyl acetate/petroleum ether, m.p. 100°, $[\alpha]_D^{25} = +0.9°$ ($c = 1$, DMF); TLC. 0.8 (M).

$C_{23}H_{28}N_2O_6$ (428.5) Calc. C 64.47 H 6.59 N 6.54% Found C 64.76 H 6.54 N 6.68%

4.2 *Z-Nva-Ser(But)-Gly-OPh.* The above protected dipeptide (7.34 g, 17.13 mmol) was hydrogenated in DMF solution using Pd/C (10%) in the presence of 1.1 equiv. HCl. Methanol was added and, after filtration from the catalyst, the solution evaporated *in vacuo*.

Z-Nva-OH (5.03 g, 20 mmol), *Bates'* reagent (12.9 g, 25 mmol) were dissolved in DMF (30 ml) then NMM (2.2 ml, 20 mmol) was added to the stirred solution at room temperature. After 10 min the above hydrogenolysis product in DMF (20 ml) was added followed by NMM (3.74 ml, 34 mmol). The reaction was stirred 4.5 h at room temperature. After the usual work-up the product was crystallised from ethyl acetate/petroleum ether to give Z-Nva-Ser(But)-Gly-OPh (6.99 g, 77%), m.p. 121–122°, $[\alpha]_D^{25} = -0.5°$ ($c = 1$, DMF); TLC. 0.7 (D).

$C_{28}H_{37}N_3O_7$ (527.6) Calc. C 63.74 H 7.07 N 7.96% Found C 63.95 H 7.14 N 7.97%

4.3 *Z-Leu-Nva-Ser(But)-Gly-OPh.* The above protected tripeptide (0.916 g, 1.74 mmol) was hydrogenated as before and reacted with *Bates'* reagent (1.16 g, 2.25 mmol), Z-Leu-OH (0.46 g, 1.7 mmol) in a mixture of acetonitrile (40 ml) and DMF (10 ml) to which NMM was slowly added (0.52 ml, 4.76 mmol). After the usual work-up the product was purified by crystallisation from ethyl acetate/petroleum ether to give Z-Leu-Nva-Ser(But)-Gly-OPh (775 mg, 80%), m.p. 198–199°, $[\alpha]_D^{25} = -11.7°$ ($c = 1$, DMF); TLC. 0.4 (B) 0.8 (M), 0.6 (D).

$C_{34}H_{48}N_4O_8$ (640.8) Calc. C 63.73 H 7.55 N 8.74% Found C 63.60 H 7.50 N 8.70%

4.4. *Z-Ala-Leu-Nva-Ser(But)-Gly-OPh* (**9**). The above protected tetrapeptide (481 mg, 0.75 mmol) was hydrogenated in the usual way and reacted with *Bates'* reagent (426 mg, 0.83 mmol), Z-Ala-OH and NMM (0.25 ml, 2.25 mmol) in DMF (10 ml). Isolation of the product by precipitation with satd. $NaHCO_3$-solution and filtration gave **9** (420 mg, 80%), m.p. 238–241°, $[\alpha]_D^{25} = -23.3°$ ($c = 1$, DMF); TLC. 0.7 (D) 0.7 (M).

$C_{35}H_{53}N_5O_9$ (711.9) Calc. C 62.43 H 7.50 N 9.84% Found C 62.34 H 7.36 N 9.57%

Amino acid analysis: acid hydrolysis (6N HCl, 24 h. 110°): Ala 0.95, Leu 0.99, Nva 1.08, Gly 0.98, Ser 0.90.

5. *Benzyloxycarbonylglycine anhydride* (**10**). Z-Gly-OH (209 mg, 1 mmol) *Bates'* reagent (774 mg, 1.5 mmol) and NMM (202 mg, 2 mmol) were dissolved in dry acetonitrile (10 ml) and the resulting solution stirred 16 h at room temperature. After removal of the solvent the residue was partitioned between water and ethyl acetate. The organic phase was washed with 2N HCl, satd. $NaHCO_3$-solution, water and brine. The solution was dried ($MgSO_4$) and concentrated to small volume whereupon **10** crystallised: (123 mg, 62%), m.p. 117–118° (ethyl acetate/petroleum ether); ν max. (nujol) 1820, 1750, 1690 cm^{-1}, identical to authentic material prepared from Z-Gly-OH using DCCI [5].

A solution of DCCI (2.06 g, 10 mmol) in acetonitrile (20 ml) was added to Z-Gly-OH (2.09 g, 10 mmol) in acetonitrile (50 ml) at – 5° and the solution was stirred 16 h at room temperature. The solution was cooled, then filtered and the filtrate worked up as above to give **10** (1.14 g, 57%), m.p. 114–117° (literature [5] m.p. 115–116°).

6. *N,N'-Bis(benzyloxycarbonyl)glycylglycine* (**11**). The anhydride **10** (1.6 g, 4 mmol) and Z-Gly-OH (0.8 g, 3.8 mmol) in benzene (20 ml) were refluxed for 4 h. After removal of the solvent *in vacuo*, the residual oil was dissolved in methanol (10 ml) and water (20 ml) added. After 16 h the solid was filtered off and washed with ether. Recrystallisation from ether/methanol/petroleum ether 100:5:25 yielded **11** (708 mg, 43%), m.p. 133–136° (literature [6]: m.p. 135–136°).

7. *[1-^{13}C] and [2-^{13}C] Z-Gly-OH.* The [^{13}C]-glycine (100 mg, 1.33 mmol) was dissolved in water (0.6 ml) and dioxan (0.2 ml). The solution was cooled to 5° and 4N NaOH (0.37 ml) was added to give pH 10.5. Benzyloxycarbonyl chloride (0.22 ml, 2.1 mmol) was added, and the pH of the solution maintained at 10.5 over 4 h by the addition of 4N NaOH (0.39 ml). The solution was cooled to – 5° and acidified to pH 2.5 with 6N HCl then left for 12 h at – 10°. Centrifugation and washing with water afforded the [^{13}C]-Z-Gly-OH. [1-^{13}C]-Z-Gly-OH (179 mg, 62%), m.p. 114–116°; [2-^{13}C]-Z-Gly-OH (198 mg, 70%), m.p. 112–116°. Both compounds have the same properties as authentic Z-Gly-OH.

8. *Bpoc-Orn(Adoc)-Thr(But)-Pro-Gly-Ser(But)-Ala-Asn-Gly-OPh.* The protected peptide Z(15)OPh (3.60 g, 5.8 mmol) was hydrogenated in DMF (100 ml) in the presence of *p*-toluene sulfonic acid monohydrate (1.11 g, 5.8 mmol) and 1 g Pd/C (5%). After 4 h the suspension was filtered and the solution concentrated *in vacuo* to *ca.* 20 ml.

Bpoc (**14**) OH (5.0 g, 5.8 mmol) was added to the stirred solution followed by *Bates'* reagent (4.5 g) and diisopropylamine (2.25 g, 17.5 mmol). After stirring for 16 h the mixture was diluted with water. The precipitated crude product was crystallised from ethyl acetate to give Bpoc (**14** + **15**)-OPh-(4.85 g, 65%), m.p. 165–167°, $[\alpha]_D^{25} = -17.1°$ (c = 1, DMF); TLC. 0.2 (D); LH20/DMF single peak ve/vt 0.41.

$C_{69}H_{96}N_{10}O_{16} \cdot H_2O$	Calc.	C 61.87	H 7.37	N 10.46%
(1339.6)	Found	,, 62.00	,, 7.60	,, 10.48%

Amino acid analysis: acid hydrolysis (6N HCl, 18 h, 110°); Asp 1.02, Thr 0.94, Ser 0.89, Pro 0.98, Gly 2.01, Ala 1.00, Orn 0.98.

9. *Bpoc-Cys(Acm)-Asn-Ile-Pro-Cys(Acm)-Ala-Ala-Leu-Nva-Ser(But)-Gly-OPh.* Bpoc(**17**)-OPh (2.12 g, 2 mmol) was dissolved in acetic acid/formic acid/water 7:1:2 (50 ml) containing dimethylsulfide(DMS) (6 ml., 80 mmol) and stirred for 2 h. The solvent was evaporated *in vacuo* and the resulting solid treated with 0.05M HCl in dioxan (80 ml, 4 mmol) containing DMS (6 ml, 80 mmol). After evaporation of the solvent *in vacuo*, the anion exchange was repeated and the solid obtained washed with ether and dried. It was then dissolved in HMPT (15 ml) containing NMM (0.22 ml, 2 mmol). This required 0.5 h stirring. Bpoc-(**16**)OH (2.23 g, 3 mmol), N-hydroxysuccinimide (HONSu) (0.69 g, 6 mmol), *Bates'* reagent (2.32 g, 4.5 mmol) and NMM (0.99 ml, 9 mmol) were added and the reaction stirred for 16 h. The solution was applied to a Sephadex LH-20 column and eluted with DMF. The fractions containing the product, vc/vt 0.41, were combined and concentrated *in vacuo* to give Bpoc(**16** + **17**)OPh (1.94 g, 62%), $[\alpha]_D^{23} = -28.8°$ (c = 2, DMF); TLC. 0.6 (C), 0.3 (H), 0.5 (M).

$C_{75}H_{110}N_{14}O_{18}S_2 \cdot 2H_2O$	Calc.	C 56.45	H 7.20	N 12.29%
(1595.9)	Found	,, 56.35	,, 7.21	,, 12.34%

Amino acid analysis: acid hydrolysis (6N HCl, 18 h 110°): Asp 0.95, Ser 0.89, Pro 0.97, Gly 1,00, Ala 2.12[6]), Nva 0.93, Ile 0.98, Leu 1.04.

10. *Bpoc-Cys(Acm)-Asn-Ile-Pro-Cys(Acm)-Ala-Ala-Leu-Nva-Ser(But)-Gly-Asp(But)-Ile-Thr-(But)-Ala-Ser(But)-Val-Gly-OPh Bpoc*(**18** + **19**)*OPh.* Bpoc(**18**)OPh (265 mg, 0.17 mmol) was dissolved DMF (7 ml) then water (1.2 ml) and DMS (0.62 ml, 8.5 mmol) added. The pH was adjusted to 10.5 with 0.1N NaOH using a pH stat. 1 equivalent of H_2O_2 was added and the steady reaction was complete in 0.5 h with base uptake 1.85 ml. The solution was cooled to −5°, acidified with 5% citric acid solution to pH 3.5 and poured into brine (15 ml). After filtration, Bpoc(**18**)OH was obtained (233 mg, 93%); TLC. 0.2 (B), 0.1 (H), 0.1 (M).

Z-(**19**)-OPh (208 mg, 0.2 mmol) and *p*-toluene sulfonic acid monohydrate (38 mg, 0.2 mmol) was dissolved in DMF (7 ml) and hydrogenated for 16 h in the presence of 50 mg Pd/C (10%). After filtration and evaporation of the solvent *in vacuo*, the *p*-toluene sulfonate of H-(**19**)OPh was obtained (196 mg, 91%); TLC. 0.6 (C), 0.7 (M).

Bpoc(**18**)OH (111 mg, 0.075 mmol), H(**19**)OPh *p*-toluene sulfonate (54 mg, 0.050 mmol), HONSu (17 mg, 0,15 mmol) and *Bates'* reagent (58 mg, 0.112 mmol) were dissolved in HMPT (2 ml). 1M NMM in DMF (0,28 ml, 0,28 mmol) was then added and the reaction stirred 16 h at room temperature. The solution was poured into brine (10 ml) and the precipitate filtered off, washed with water, propan-2-ol, ether and dried to give Bpoc-(**18** + **19**)-OPh (98 mg, 83%).

Amino acid analysis: acid hydrolysis (6N HCl, 18 h, 110°): Asp 2.04, Thr 0.96, Ser 1.84, Pro 0.97, Gly 2.03, Ala 3.23, Val 1.13, Nva 0.92, Ile 1.95, Leu 1.00.

$C_{114}H_{181}N_{21}O_{24}S_2 \cdot 5H_2O$	Calc.	C 55.57	H 7.81	N 11.94%
(2464,0)	Found	,, 55.80	,, 7.82	,, 11.61%

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81. *Ab initio* SCF Calculation of the Fluoronium Ion: Geometry, Electronic Structure and Vibrational Constants

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Summary. An *ab initio* SCF calculation of 42 points of the energy hypersurface of the fluoronium ion is presented using a contracted F(5*s*/3p), H(2*s*) gaussian basis set. In its equilibrium structure a bond length of 1.812 a.u. and a HFH bond angle of 127.2° are predicted. The calculated vibrational frequencies for H_2F^+, HDF^+, and D_2F^+ are in good agreement with the experimental data.

1. Introduction. – The vibrational spectra of the fluoronium ions H_2F^+, HDF^+, and D_2F^+ have recently been reported by *Couzi et al.* [1]. They concluded that the molecules are bent in their equilibrium configuration and derived, by assuming HFH angles between 105° and 120°, sets of harmonic force constants. *Pople et al.* [2] employed minimal STO-3G and extended 4-31G basis sets in a systematic *ab initio* study of the geometries and energies of AH_n molecules and cations. The fluoronium ion H_2F^+, having the same electron configuration as H_2O, was predicted to have a somewhat larger bond angle than water. *Leibovici* [3] investigated the structure of H_2F^+ and the path of protonation of HF using semiempirical and *ab initio* calculations. The activation energy of protonation is thought to result from solvation effects only.

An understanding of rotational and virbational energy transfer and of chemical reactivity can be obtained by calculating energy hypersurfaces and solving the collision dynamical problem by trajectory calculations. The description of the hypersurfaces in analytical form over an extended range, however, presents serious problems. *Schaefer et al.* [4] determined the interaction potential between two rigid HF molecules in