Design and Synthesis of Multidetachable Resin Supports for Solid-Phase Peptide Synthesis

James P. Tam,* Foe S. Tjoeng, and R. B. Merrifield

Contribution from The Rockefeller University, New York, New York 10021.
Received March 12, 1980

Abstract: A new and unusually versatile class of supports for solid-phase peptide synthesis has been developed. It contains two orthogonally removable ester bonds, allowing the selective cleavage of the individual bonds by a variety of reagents. The synthetic peptide can be obtained in a free or protected form, or with a removable spacer group still attached at the carboxyl terminus. Two such multidetachable resin supports have been developed in which selective cleavage is by acidolysis, photolysis, or nucleophilic cleavage; they are (1) *tert*-butoxycarbonylaminoacyl-2-[4-(oxymethyl)phenylacetoxy]propionyl-copoly(styrene–divinylbenzene) resin (Pop-resin) and *tert*-butoxycarbonylaminoacyl-4-[4-(oxymethyl)phenylacetoxymethyl]-3-nitrobenzamidomethyl-copoly(styrene–divinylbenzene) resin (Pon-resin). The value of these resins was demonstrated by the stepwise synthesis of Leu-enkephalin and angiotensin II. In addition, applications of this class of supports for fragment synthesis were described. The minimization of side reactions associated with peptide anchored to photolabile resins in the ordinary way was demonstrated.

Most peptide-resin attachments for solid-phase synthesis to date have been designed for a single specific purpose.[1-10] They each contain only one labile anchoring bond, are generally cleavable by only one or a few related methods, and are intended for use in only one synthetic strategy. Thus, the first system, and its subsequent modifications, contained a benzyl ester anchoring bond and was designed for stepwise synthesis and cleavage in strong acid. Subsequent modifications led to systems in which the benzylic anchoring bond was more stable to acidolysis[5-7] and to others in which it was much more susceptible to cleavage under mild acid conditions.[8] Greater chemoselectivity between the protecting groups of the α-amine, the side chains, and the anchoring bond was achieved by the development of resins that can be cleaved with base,[9] nucleophiles,[4] or photolysis.[2,3] Although these modifications have been very useful, they nevertheless have been single-purpose systems.

We report here the design and synthesis of new multipurpose and multidetachable resin supports based on a new concept that provides much more flexibility in synthetic design than has been possible before.[11]

Concept and Development of a Multidetachable Support

The basic idea makes use of an attachment, peptide–O–X–O–Y–resin, with two orthogonal[12] ester bonds, bond a (peptide–O–X) and bond b (X–O–Y), separated by a suitable spacer (X) (Scheme I). Thus, depending on the reagent, one or the other of the esters can be cleaved selectively under conditions where the second bond is stable, and the peptide can be obtained in a deprotected form

Scheme I. A Design of Multidetachable Resins

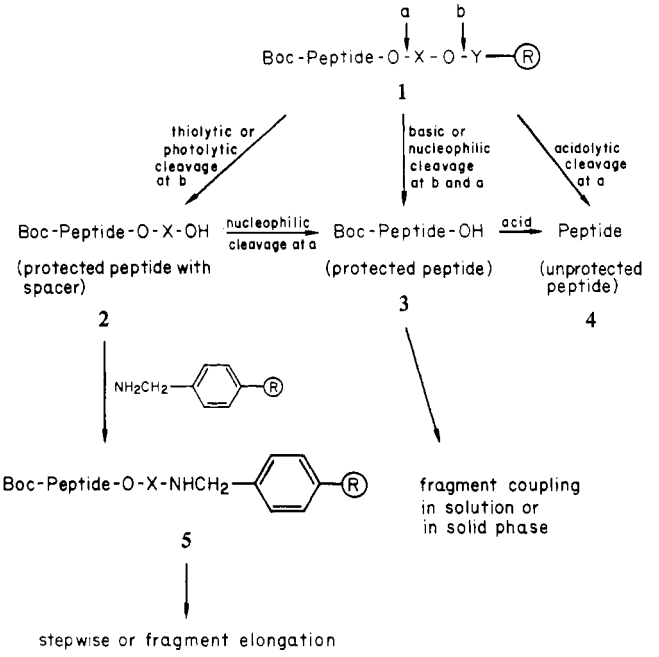


Table I. Orthogonal Cleavages of Multidetachable Peptide-Resins[a]

	cleavage condition	starting point	cleavage point	product
1	strong acid (HF, $MeSO_3H$)	1, 2	a	4
2	photolysis	1	b	2
3	nucleophile:			
	(a) cyanolysis	1	b, a	3
	(b) thiolysis	1	b	2
4	hydrogenolysis	1, 2	a	3
5	base	1	b, a	3
6	H_2NNH_2	1	b, a	3[b]
7	mild acid (TFA)	3		4

[a] See Scheme I. [b] Boc-peptide-hydrazide.

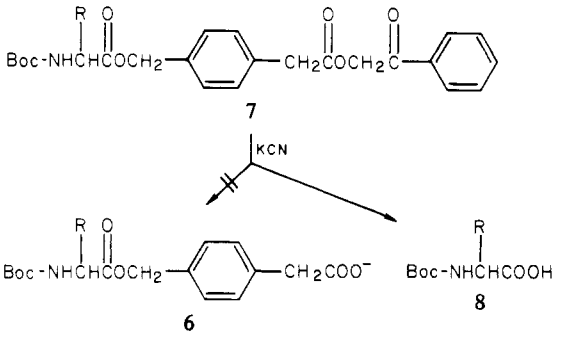
4, in a protected form **3**, which is suitable for fragment synthesis, or in an intermediate form **2**, which can be reattached to another resin support (e.g., an aminomethyl resin) to give **5** for stepwise or fragment elongation. Furthermore, the system is designed so that a mild interconversion from one form to the other can be effected (Table I).

Of the several orthogonally removable esters that were considered to implement the general plan, the best combination was

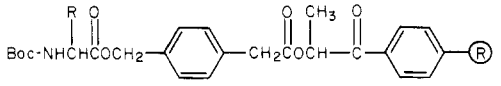
(1) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2145 (1963).
(2) D. H. Rich and S. K. Gurwara, *J. Am. Chem. Soc.*, **97**, 1575 (1975).
(3) S. S. Wang, *J. Org. Chem.*, **41**, 3258 (1976); J. C. Sheehan and K. Umezawa, *ibid.*, **38**, 3771 (1973).
(4) T. Mizoguchi, K. Shigezane, and N. Takamura, *Chem. Pharm. Bull.*, **17**, 411 (1969); **18**, 1465 (1970); J. T. Sparrow, *J. Org. Chem.*, **41**, 1350 (1976).
(5) A. R. Mitchell, B. W. Erickson, M. N. Ryabtsev, R. S. Hodges, and R. B. Merrifield, *J. Am. Chem. Soc.*, **98**, 7357 (1976).
(6) A. R. Mitchell, S. B. H. Kent, M. Engelhard, and R. B. Merrifield, *J. Org. Chem.*, **43**, 2845 (1978).
(7) C. H. Li, D. Yamashiro, L. F. Tseung, and H. H. Loh, *J. Med. Chem.*, **20**, 325 (1977); J. T. Sparrow, *J. Org. Chem.*, **41**, 1350 (1976).
(8) S. S. Wang and R. B. Merrifield, *Int. J. Pept. Protein Res.*, **4**, 309 (1972); G. L. Southard, G. S. Brooke, and J. M. Pettee, *Tetrahedron Lett.*, 3505 (1969).
(9) G. W. Kenner, J. R. McDermott, and R. C. Sheppard, *J. Chem. Soc. D*, 636 (1971).
(10) P. Pietta and O. Brenna, *J. Org. Chem.*, **40**, 2995 (1975); P. Pietta and G. R. Marshall, *J. Chem. Soc. D*, 650 (1970).
(11) J. P. Tam, F. S. Tjoeng, and R. B. Merrifield, Proceedings of the 6th American Peptide Symposium, 1979; E. Gross, J. Meienhofer, and R. Vigna, Ed., Pierce Chemical Co. (Ill.), pp 341–344; J. P. Tam, F. S. Tjoeng, and R. B. Merrifield, *Tetrahedron Lett.*, 4935–4938 (1979).
(12) The term orthogonal was used to denote a chemoselectively removable protecting group: D. S. Kemp, Fifth American Peptide Symposium, New York, 1975; G. Barany and R. B. Merrifield, *J. Am. Chem. Soc.*, **99**, 7363 (1977).

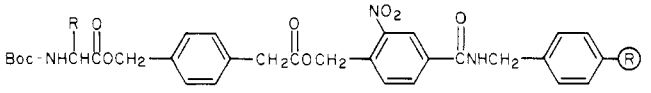
0002-7863/80/1502-6117$01.00/0

Scheme II. Conversion of *tert*-Butoxycarbonylaminoacyl-4-oxymethylphenylacetic Acid Phenacyl Ester to Boc Amino Acid by Treatment with KCN

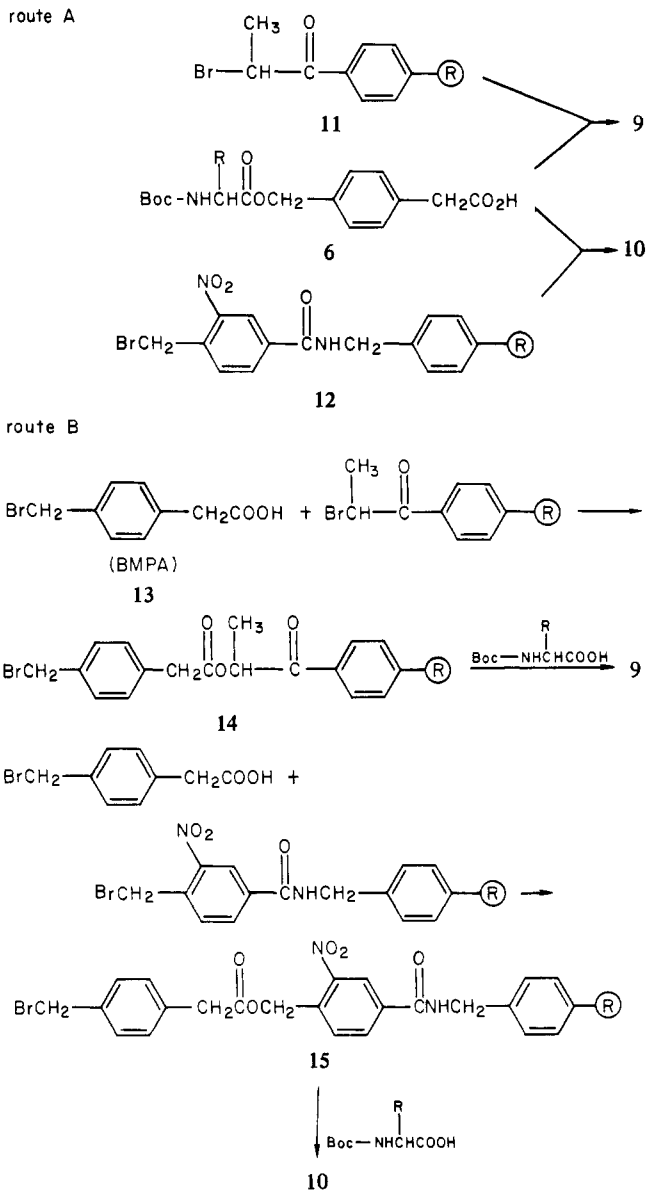


found to be an acid-labile benzyl ester at bond a and a photolabile α-methylphenacyl ester or *o*-nitrobenzyl ester at bond b. The former is stable to photolysis and the latter are stable to acidolysis. With this combination **2** can be generated by cleavage at bond b, and **3** by cleavage at bond a. The problem was to select the intermediate spacer X such that **2** could be converted to **3** without loss of other protecting groups. Spacers that require cleavage by strong acid or by hydrogenolysis would not completely fulfill the requirements because most of the side-chain benzyl groups would be lost. The alternative of placing the benzyl ester at bond b and the photolabile ester at bond a also would not solve the problem because a direct acidolytic pathway from **1** to **4** would not be possible. Fortunately, a clue to the problem came during the preparation of *tert*-butoxycarbonylaminoacyl-4-oxymethylphenylacetic acid (Boc-aminoacyl-OMPA, **6**)[13a] needed for the synthesis of Pam-resin.[6,14] The intermediate Boc-aminoacyl-OMPA phenacyl ester **7** was found upon treatment with cyanide ion[13b] to give Boc amino acid **8** directly instead of the expected oxymethylphenylacetic acid derivative **6** (Scheme II). Similar transformation of **7** to **8** was also observed in aqueous base and nonnucleophilic amine base such as 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU), but not in triethylamine or diisopropylethylamine. However, the oxymethylphenylacetic acid derivative **6**, like the ordinary benzyl ester, is relatively stable to such treatment.[13,15] The *tert*-butoxycarbonylaminoacyl-4-oxymethylphenylacetic acid phenacyl ester **7** provided a reasonable solution model for the required new resin support and the missing piece for the spacer X. Thus the new resins selected for further study were *tert*-butoxycarbonylaminoacyl-2-[4-(oxymethyl)phenylacetoxy]propionyl-resin (Boc-aminoacyl-OCH_2-Pop-resin, **9**) and *tert*-butoxycarbonylaminoacyl-4-[4-(oxymethyl)phenylacetoxymethyl]-3-nitrobenzamidomethyl-resin (Boc-aminoacyl-OCH_2-Pon-resin, **10**). In each case the resin was a copolymer of styrene and 1% divinylbenzene.





Scheme III. Preparation of Pop- and Pon-Resins



Synthesis of the Multidetachable Pop- and Pon-Resins

The Pop- and Pon-resins **9** and **10** were prepared in two ways (Scheme III): route A, by direct esterification of Boc-aminoacyl-OMPA to either 2-bromopropionyl-resin **11**[3] or 4-bromomethyl-(3-nitro)benzamidomethyl-resin **12**;[2] or route B, by first selectively esterifying bromomethylphenylacetic acid (**13**) to either 2-bromopropionyl-resin or 4-bromomethyl-(3-nitro)benzamidomethyl-resin to give **14** and **15** and then acylating with the Boc amino acid to produce **9** and **10**.

Route A (Scheme III) is well defined and is the preferred way to prepare either Pop- or Pon-resin, since the soluble intermediates can be isolated and purified before attachment to the resin. The first step, leading to Boc-aminoacyl-OMPA (**6**), has already been worked out in the earlier studies on Boc-aminoacyl-OCH_2-Pam-resin.[6,14] The esterification of Boc-aminoacyl-OMPA to both photolabile resins **11** and **12** could be achieved by the Cs salt method,[16] but was more conveniently and reproducibly achieved by using potassium fluoride[17] in *N*-methylpyrrolidone as solvent.[14,18] Under these conditions total esterification was obtained

(13) (a) Abbreviations used in the text: BMPA, bromomethylphenylacetic acid; Boc, *tert*-butoxycarbonyl; DBU, 1,5-diazabicyclo[5.4.0]undec-5-ene; DCC, dicyclohexylcarbodiimide; DIEA, diisopropylethylamine; DMF, dimethylformamide; HOBt, hydroxybenzotriazole; NMP, *N*-methylpyrrolidone; OMPA, oxymethylphenylacetic acid; Pac, phenacyl; Pam, phenylacetamidomethyl; Pon, 4-(phenylacetoxymethyl)-3-nitrobenzamidomethyl; Pop, 2-(phenylacetoxy)propionyl; TFA, trifluoroacetic acid. (b) J. P. Tam, W. F. Cunningham-Rundles, B. W. Erickson, and R. B. Merrifield, *Tetrahedron Lett.*, 4001 (1977).

(14) J. P. Tam, S. B. H. Kent, T. W. Wong, and R. B. Merrifield, *Synthesis*, **12**, 955 (1979).

(15) J. P. Tam, F. S. Tjoeng, and R. B. Merrifield, manuscript in preparation.

(16) B. F. Gisin, *Helv. Chim. Acta*, **56**, 1476 (1973).

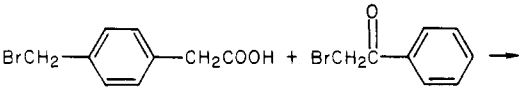
(17) J. H. Clark, H. L. Holland, and J. M. Miller, *Tetrahedron Lett.*, 3361 (1976); J. H. Clark and J. M. Miller, *J. Chem. Soc., Chem. Commun.*, 229 (1976).

(18) J. P. Tam, F. S. Tjoeng, and R. B. Merrifield, manuscript in preparation.

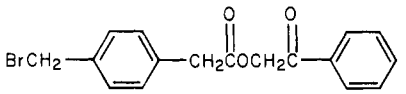
in a shorter time, at lower temperature, and without discoloration of the resin. Esterification of Boc-Leu-OMPA and Boc-Val-OMPA by **11** was quantitative after 24 h at room temperature, while Boc-Gly-OMPA and Boc-Phe-OMPA required 50 °C for complete reaction in that time.

Synthesis by route B (Scheme III) was simpler to carry out and in general is a more flexible approach since all amino acids can be introduced from a single intermediate for each resin (**14** or **15**). This approach was possible for two reasons: (1) our earlier experimental data had shown that phenacyl bromide (a solution model of resin **11**) reacted much faster than benzyl bromide with Boc amino acid salts, and therefore the side reaction due to dimerization of bromomethylphenylacetic acid (BMPA, **13**) to bromomethylphenylacetoxymethylphenylacetic acid (BMPA-OMPA) and its subsequent reaction with resin **11** or **12** to form the dimeric BMPA-OMPA-resin products would be expected to be minor; (2) even if these byproducts were formed, small amounts should not be harmful to the overall reactions in the scheme since the extra oxymethylphenylacetyl group would be removable either by the strong acid cleavage route or by the basic cleavage route and the same product would be obtained.

To effect the esterification of BMPA (**13**) to either **11** or **12**, various conditions were examined using **13** + **16** to **17** as a soluble



13 **16**



17

model. The progress of the reaction could be monitored readily by following the ester peak in the IR at 1720 cm^{-1}. For the model reaction, other esterification methods such as potassium salt/crown ether complex,[19] $CsHCO_3$,[16] and tertiary amine[19] methods were found to be less satisfactory than the potassium fluoride method.[18] The distribution of products after esterification to the resin by the KF method was found to be solvent dependent. The rates of both dimerization and esterification increased with more polar aprotic solvents and were fastest in DMF, which is a good resin-swelling solvent. CH_3CN, a poor swelling solvent, promoted esterification within 2 h and dimerization was not observed in 6 h. The best condition was found to be the mixed solvent *N*-methylpyrrolidone–acetonitrile–dioxane (1:4:4), 50 °C, for 12 h. The formation of dimer was determined to be less than 5%.

The esterification of Boc amino acid to the bromomethyl-Pop-resin **14** or bromomethyl-Pon-resin **15** could also be easily achieved in KF, using NMP as solvent. Again, as with the OMPA derivatives, Boc-Val-OH and Boc-Leu-OH could be esterified in 24 h at room temperature while Boc-Phe-OH and Boc-Gly-OH required 50 °C to achieve the same rate.

However, esterification using either route A or B on prolonged heating with base often led to undesirable results in which the properties of these resins were altered to give diminishing efficiency of the multidetachable resins and decreased the amount of loading yields of Boc amino acids.[15] Further efforts to prepare this resin by using hydroxymethylphenylacetic acid or chloromethylphenylacetic acid for BMPA (**13**) are being pursued. These two intermediates should offer more selectivity and thus better esterification to the photolabile resins **11** and **12**.

Susceptibility of the Ester Bonds of Boc-Aminoacyl-OCH_2-Pop-Resins and Boc-Aminoacyl-OCH_2-Pon-Resins by Various Reagents

A. Strong Acid. Four Boc amino acids were anchored by route A onto the Pop-resin and two onto the Pon-resin. These resins were subjected to strong acid to effect cleavage of the benzyl ester (bond a, Figure 1). As shown in Table II, the yields ranged from 80 to 90% with either HF[20,21] or CH_3SO_3H.[22] Cleavages were essentially the same from the Pop-resin and the Pon-resin since the bond being cleaved is the same and is far removed from the point of difference in the two supports. There was also little difference between the cleavage yields of different amino acids, dipeptides, or tetrapeptides.

Table II. Acidic Cleavage of Multidetachable Resins

sample[a]	cleavage yield, %[b] HF[c]	$MeSO_3H$[d]
Boc-Gly-OCH_2-Pop-resin (**9a**)	84	87
Boc-Val-OCH_2-Pop-resin (**9b**)	89	88
Boc-Leu-OCH_2-Pop-resin (**9c**)	89	86
Boc-Phe-OCH_2-Pop-resin (**9d**)	82	80
Boc-Gly-OCH_2-Pon-resin (**10a**)	86	85
Boc-Val-OCH_2-Pon-resin (**10b**)	80	81
Boc-Leu-Phe-OCH_2-Pop-resin (**9e**)	86	82
Boc-Pro-Gly-OCH_2-Pop-resin (**9f**)	93	80
Boc-Leu-Ala-Gly-Val-OCH_2-Pop-resin (**9g**)	91	
Boc-Leu-Ala-Gly-Val-OCH_2-Pon-resin (**9h**)	86	

[a] Prepared by route A. [b] Based on amino acid analysis; (μmol released from resin/μmol on resin before treatment) × 100. [c] 0.5 h, 0 °C, 4.5 mL of HF, 0.5 mL of anisole. [d] 0.5 h, 0 °C, MSA/TFA/CH_2Cl_2/anisole (5:2:2:1).

Table III. Acid Stability of Boc-aminoacyl-OCH_2-Pop-Resins (**9a–d**) in Refluxing TFA

resin	k, 10^{-6} s^{-1} [b]	% loss/min	k_{rel}
Boc-Val-OCH_2-resin[a]	717	4.2	[100]
Boc-Val-OCH_2-Pam-resin[a]	5.1	0.031	0.7
Boc-Val-OCH_2-Pop-resin	7.8	0.047	1.1
Boc-Gly-OCH_2-Pop-resin	20.7 (7.4)[c]	0.120	2.9 (1)[c]
Boc-Leu-OCH_2-Pop-resin	10.3	0.062	1.4
Boc-Phe-OCH_2-Pop-resin	3.7 (3.6)[c]	0.022	0.5 (0.5)[c]

[a] Taken from ref 12. [b] Apparent first-order constants were determined from plots of ln $[a/(a-x)]$ vs. time where a is the amino acid content of the starting material and x is the amount of acid released at a given time. [c] Values of corresponding amino acid derivative attached to the Pam-resin from ref 12.

The cleavage yields from Boc-aminoacyl-OCH_2-Pop- or -Pon-resins that had been prepared by route B were not as high as from those prepared by route A. We attribute this to the presence of small amounts of *tert*-butoxycarbonylaminoacyloxy-2-propionyl-resin or *tert*-butoxycarbonylaminoacyloxy(3-nitro)-benzyl-resin, which are stable to acidolysis. These products could arise by a failure to completely cover all of the 2-bromopropionyl-resin or 4-bromo-3-nitrobenzamido-resin with the bromomethylphenylacetic acid. Subsequent photolytic cleavage at bond b (Figure 1) substantiated this conclusion.

B. Trifluoroacetic Acid. The Boc-aminoacyl-OCH_2-Pop-resins (**9a–d**) containing Gly, Val, Phe, and Leu were treated with refluxing TFA. The rates were determined by the loss of amino acid, which followed first-order kinetics for each of the resins. As shown in Table III the Boc-aminoacyl-OCH_2-Pop-resins (**9a–d**) are 35–200-fold more stable than Boc-Val-OCH_2-resin in refluxing trifluoroacetic acid. As expected, they have acid stabilities similar to those of the corresponding Boc-aminoacyl-OCH_2-Pam-resins[6] which contain the 4-acetamido spacer rather than the 4-acetoxy spacer as in the Pop-resin. Thus, extrapolating to the normal synthetic cycles of solid-phase synthesis, the loss of peptide chain due to acidolytic cleavage in a 20-min deprotection step is 0.004–0.02%/cycle as compared to 0.78% for the conventional oxymethyl-resin.[6] As in the case of the Pam-resin this enhanced

(19) K. Suzuki and N. Endo, *Chem. Pharm. Bull.*, **25**, 1143 (1977); R. W. Roeske and P. D. Gesellchen, *Tetrahedron Lett.*, 3369 (1976).

(20) S. Sakakibara and Y. Shimonishi, *Bull. Chem. Soc. Jpn.*, **38**, 1412 (1968).

(21) J. Lenard and A. B. Robinson, *J. Am. Chem. Soc.*, **89**, 181 (1967).

(22) H. Yajima, Y. Kiso, H. Ogawa, N. Fujii, and H. Irie, *Chem. Pharm. Bull.*, **23**, 1164 (1975).

Table IV. Cleavage Methods for Multidetachable Resins

resin[a]	cleavage yields, %[b]							
	photolysis	Bu_4N-CN	KCN/crown ether	Et_3N/PhSH	OH^- [c]	H_2NNH_2 [d]	H_2	cyclohexadiene[e]
Boc-Gly-OCH_2-Pop-resin (**9a**)	86	84	96	92	84	87	70	78
Boc-Val-OCH_2-Pop-resin (**9b**)	80	69	71	78	71	92	50	66
Boc-Leu-OCH_2-Pop-resin (**9c**)	79	75	78	85	75	86	63	72
Boc-Phe-OCH_2-Pop-resin (**9d**)	82	80	79	81	80	85	65	65
Boc-Gly-OCH_2-Pon-resin (**10a**)	83				81	88	66	70
Boc-Val-OCH_2-Pon-resin (**10b**)	80				71	87	56	65
Boc-Leu-Phe-OCH_2-Pop-resin (**9e**)	81	69			80			
Boc-Pro-Gly-OCH_2-Pop-resin (**9f**)	83	80						
Boc-Leu-Ala-Gly-Val-OCH_2-Pop-resin (**9g**)	75	78			78			
Boc-Leu-Ala-Gly-Val-OCH_2-Pon-resin (**9h**)	72				80			

[a] Prepared by route A. [b] Yield based on amino acid analysis; (μmol released from resin)/(μmol on untreated resin) × 100. [c] Triton B/dioxane/water (1:5:5). [d] Hydrazine/DMF. [e] Transfer hydrogenation in $Pd(OAc)_2$.

acid stability is highly desirable in order to reduce the loss of peptide chains from the support and to prevent the formation of shorter peptides due to late initiation of chains on the resulting hydroxymethyl sites[23] or termination of peptide chain by trifluoroacetylation.[23] Furthermore, this increased stability of the resin to trifluoroacetic acid can be achieved without sacrificing the high cleavage yields obtained by treatment with anhydrous HF (Table II).

C. Photolysis. The versatility of the Pop- and Pon-resins **9** and **10** was demonstrated by their cleavage with reagents other than acid (Table III). Photolysis of bond b (Figure 1) at >350 nm for 72 h in DMF gave yields ranging from 75 to 91%. This is a significant improvement over previous photolyzable bonds where yields have been lower and have been dependent on the structure of the C-terminal amino acid residue. It has been reported that only glycyl-resins have given good yields.[2,3] The improved yields with other residues in the new system can be attributed to the fact that the reactive photolabile center is between the oxymethylphenylacetic acid and the α-methylphenacyl-resin or 3-nitrobenzyl-resin and therefore is removed from the amino acid residue itself. The products obtained from these cleavages are the *tert*-butoxycarbonylaminoacyl-4-oxymethylphenylacetic acids corresponding to the various amino acids or peptides, rather than the free amino acid or peptide. These products have all been stable to further photoreactions at these wavelengths. Thin layer and ion-exchange chromatography revealed less than 1% of the Boc amino acids after prolonged photolysis.

D. Basic and Nucleophilic Reagents. Results of the cleavage of these resins by aqueous base and nucleophiles such as Bu_4NCN, KCN/crown ether, hydrazine, or thiophenoxide are shown in Table IV. The a ,ueous base, a mixture of Triton B, water, and dioxane in the ratio of 1:3:6, was quite effective in the cleavage reaction to give Boc amino acids directly.[24] A more facile basic cleavage was by the use of the nonnucleophilic amine diazabicyclo-[5.4.0]undec-5-ene (DBU). Quantitative yields of Boc amino acids were obtained in 1–2 h when **9a–d** were treated with 2–3 equiv of DBU in DMF. Tertiary amines such as triethylamine or diisopropylethylamine in DMF were totally ineffective.

Earlier we reported the use of KCN/crown ether in the selective cleavage of protected peptide fragments from phenacyl ester resins[13,15] and now we find that the cyanolytic cleavage of the Pop-resin by KCN/crown ether is also fast and quantitative. Tetrasubstituted ammonium cyanide salts such as Bu_4NCN are also effective in the cleavage of this kind of resin to give Boc amino acids directly. Bu_4NCN, unlike KCN, is extremely soluble in most organic solvents, but the reaction only proceeded at a satisfactory rate in aprotic dipolar solvents such as dimethylformamide or *N*-methylpyrrolidone. The cleavage occurs in 2–4 h at room temperature with a 1 M concentration of reagent.

In contrast to basic and cyanolytic cleavage, the thiolytic cleavage effected by thiophenoxide was slower and gave the OMPA derivative as the product. For example, Boc-aminoacyl-OCH_2-Pop-resins **9a–d** gave only 30–35% yield in 4 h and about 50% in 24 h, and only after prolonged treatment of more than 48 h could the Boc amino acid OMPA derivative be quantitatively cleaved. The thiophenoxide was generated in situ by adding the appropriate amount of Et_3N. This procedure was more convenient and reproducible than one using 1 M sodium thiophenoxide in DMF for 60 h.[25] The cleavage of Pop- or Pon-resins by hydrazinolysis was extremely facile and was usually complete in 1 h, with yields of 85–92%. In the presence of a large excess of hydrazine, *tert*-butoxycarbonylaminoacylhydrazide was liberated as the product.

Thus, multidetachable resins when treated with appropriate basic or nucleophilic reagents can yield any one of several reaction products. Boc amino acids are obtained after both cyanolytic and basic treatment, while the corresponding hydrazides are the products of hydrazinolysis. In a milder nucleophilic cleavage, thiolysis, *tert*-butoxycarbonylaminoacyl-4-oxymethylphenylacetic acids are obtained.

E. Hydrogenolysis. The reports by several laboratories that peptides can be released from the benzyl ester resin by hydrogenolysis using palladium(II) acetate in DMF at 50–70 °C with different hydrogen sources (hydrogen gas[26] or transfer hydrogenation conditions with 1,4-cyclohexadiene)[27] led us to explore this system to cleave peptides from the Pop- or Pon-resins under mild conditions. We have envisioned that this method can avoid several serious side reactions found with HF cleavage. Furthermore, protected peptide fragments can be obtained this way with minimal side-chain protecting groups to increase their solubility for large peptide coupling. In view of these advantages, and the fact that the phenacyl anchoring bond is fairly resistant to hydrogenolytic conditions, we have carried out experiments using both hydrogen sources (Table IV). Resins **9a–f** with palladium(II) acetate in DMF at 50 °C using H_2 gas as hydrogen source as reported by Schlatter, Mazur, and Goodmonson[26] gave 50–70% of Boc amino acids in 16–24 h. Similarly, the use of transfer-hydrogenation conditions as reported by Khan and Sivanandaiah[27] and Felix et al.[27] gave yields of 65–78%. More reproducible results could be obtained if a large excess (>10 equiv) and high concentration (1 M) of palladium(II) acetate were used. Hydrogenation of Boc-Leu-enkephalin-OCH_2-Pop-resin and Boc-Leu-Ala-Gly-Val-OCH_2-Pon-resin yielded 55 and 61% yields, respectively, in 18 h at 50 °C using transfer-hydrogenation conditions with 1,4-cyclohexadiene as hydrogen source.

(23) S. B. H. Kent, A. R. Mitchell, M. Engelhard, and R. B. Merrifield, *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 2180 (1979).

(24) C. Birr, M. Wengert-Müller, and A. Buku, "Peptides, Proceedings of the Fifth American Peptide Symposium", M. Goodman and J. Meienhofer, Eds., Ann Arbor Science Publishers, Ann Arbor, Mich., 1977, pp 510–513.

(25) J. C. Sheehan and G. D. Davies, Jr., *J. Org. Chem.*, **29**, 2006 (1964).

(26) J. M. Schlatter, R. M. Mazur, and O. Goodmonson, *Tetrahedron Lett.*, 2851 (1977); D. A. Jones, Jr., *Tetrahedron Lett.*, 2853 (1977).

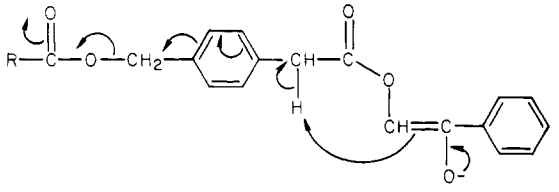
(27) S. A. Khan and K. M. Sivanandaiah, *Synthesis*, 750 (1978); A. M. Felix, E. P. Heimer, T. J. Lambros, C. Tzongraki, and J. Meienhofer, *J. Org. Chem.*, **43**, 4194 (1978).

F. Primary and Tertiary Amines. The base lability of resins **9a–d** to a primary amine was tested by treatment with 1 M benzylamine in CH_2Cl_2 at room temperature for 1 h. The losses of amino acids were calculated from the amino acid analysis of the remaining resin. All four resins gave high recovery yields of 97–99%, indicating that there was little cleavage of either ester bond by the primary amine. Therefore, in the normal coupling situation, loss due to inter- or intramolecular aminolysis by the N^{α}-amino group of the peptide chain should not pose a problem. Treatment of resins **9a–d** with 5% triethylamine in CH_2Cl_2 for 24 h at room temperature caused no loss of Boc amino acids. The base stability of the multidetachable resins in the normal protocol of stepwise solid-phase peptide synthesis was further verified in the synthesis of Leu-enkephalin, where greater than 99% of the growing chains were retained after four synthetic cycles.

Conversion of *tert*-Butoxycarbonylaminoacyl-4-oxymethylphenylacetic Acid

When Boc-aminoacyl-OCH_2-Pop-resins **9a–d** were treated with KCN with or without crown ether in dimethylformamide, Boc amino acids were obtained instead of the corresponding Boc-aminoacyl-OMPA derivatives. In order to investigate the mechanistic aspects of this facile transformation, Boc-Val-OMPA-phenacyl ester **7** (–OPac) was used as the solution model. Thus, when **7** was treated with unsolvated cyanide ion, it produced a reddish color and instantaneously converted into Boc-Val-OH (**8**) (Scheme II), with no detectable amount of Boc-Val-OMPA (**6**) present. Under the same conditions, the corresponding Boc-Val-OBzl, either as a free solution model derivative or attached onto the polymeric support, decomposed very slowly ($t_{1/2}$ > 150 h) to Boc-Val-OH. Because of the great difference in rate ($k_{rel} \gg 6000$) and the multiplicity of aromatic products obtained from the reaction of **7**, a mechanism other than direct S_N2 displacement by the cyanide anion to Boc-Val-OH is likely.

Initially, our observation that phenacyl esters of Boc amino acids were also instantaneously converted to their corresponding Boc amino acids by unsolvated cyanide in DMF led to our speculation that Boc-Val-OMPA-OPac (**7**) or Boc-Val-OCH_2-Pop-resin (**9b**), was first converted to its OMPA salt by the S_N2 displacement of the phenacyl ester and then followed by a decarboxylative 1,6 elimination to Boc-Val-OH. However, it has been found that Boc-aminoacyl-OMPA (**6**), or its salt, is converted only slowly to the Boc amino acid **8** by cyanide in aprotic solvents such as DMF ($t_{1/2}$ > 12 h), and therefore **6** is unlikely to be an intermediate in the reaction. A possible mechanism is an attack of the phenacyl enolate of **18** on its phenylacetyl methylene proton followed by a 1,6 elimination to **8**. This route is supported by



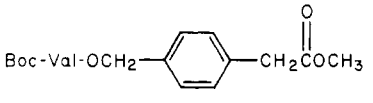
18

the following information:

1. Conversion of Boc-aminoacyl-OMPA (**7**) to the Boc amino acid by cyanide only occurred readily in aqueous alcohol ($t_{1/2}$ < 3 min), not in aprotic solvents.

2. The possible side product of cyanolytic cleavage of **7**, phenacyl cyanide, did not accelerate the cleavage of **6** in DMF.

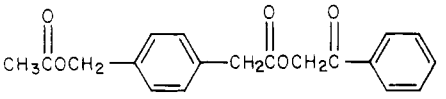
3. Triethylamine or diisopropylethylamine did not transform **7** or **19**.



19

4. Cyanide or DBU treatment of **19** in DMF did not result in any measurable reaction after 1 h.

5. Systems based on 1,6 elimination are known[28-31] and are used to generate *p*-xylylene and to prepare[2.2]paracyclophane.[28] The mechanism by which **7** was converted to its OMPA salt **8**, followed by a decarboxylative 1,6 elimination, would have generated this reactive *p*-xylylene. Attempts to identify [2.2]paracyclophane to implicate the presence of *p*-xylylene in the reaction mixtures of compounds **9a–d** with tetrabutylammonium cyanide failed. In addition, paracyclophane could not be detected after reaction of *tert*-butoxycarbonylvalyloxymethylphenylacetic acid phenacyl ester (**7**) or the simpler model compound **20** with DBU.



20

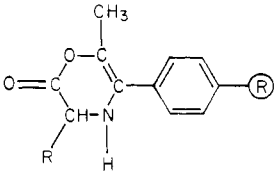
6. NMR deuterium exchange experiments of **7** with DBU in $DCCl_3$, where the elimination reaction was slow, showed the rapid disappearance ($t_{1/2} \sim 3$ min) of both sets of singlets at 5.12 (OCH_2COPh) and 3.64 ppm ($-PhCH_2CO-$), while the methylene singlet at 3.60 ppm ($-PhCH_2CO_2CH_3$) of the analogous compound **19** showed little change under the same conditions.

Test for Racemization

Phenylalanine dipeptides are known to be prone to racemization. Therefore, Boc-Leu-Phe-OCH_2-Pop-resin (**9e**) was prepared by esterification of Boc-Leu-Phe-OMPA with resin **11** and then used as a model for racemization tests. The dipeptide was cleaved from the resin by several of the methods described above and, where necessary, treated with 50% TFA/CH_2Cl_2 to remove the Boc group. Photolysis of **9e** gave Boc-Leu-Phe-OMPA (elution time 662 min), which was deprotected by TFA/CH_2Cl_2 and then by hydrogenolysis. The resulting Leu-Phe was analyzed by ion-exchange chromatography to separate the L-Leu-L-Phe and L-Leu-D-Phe diastereomers.[32] No racemization was observed in the three cleavage methods that were tested (HF, photolysis, and cyanolysis). The test was sensitive enough to have detected 0.1% of the D-Phe isomer. This result shows that peptide fragments containing C-terminal residues other than glycine or proline can be prepared on the Pop resin without danger of racemization during the cleavage step.

Avoidance of Side Reactions Associated with Phenacyl Resins

A major impetus to the development of this new class of resin was our previous observation of significant losses of α-amino groups during the first two cycles of synthesis of peptides using ordinary anchoring to phenacyl resins.[33] These losses were sometimes as high as 50%. They were attributed to (a) loss of the first amino acid due to cyclization by reaction of the N^{α}-amino group with the ketone of the phenacyl group to form a dihydrooxazinone **21**



21

and (b) loss of the first two residues due to formation of a diketopiperazine, which is promoted by the good phenacyl leaving

(28) D. J. Cram and J. M. Cram, *Acc. Chem. Res.*, **4**, 204 (1977).
(29) D. S. Kemp and C. F. Hoyng, *Tetrahedron Lett.*, 4625 (1975); D. S. Kemp and D. C. Roberts, *ibid.*, 4629 (1975); D. S. Kemp and J. Reczek, *ibid.*, 1031 (1977).
(30) M. Wakselman and E. Guibe-Jampel, *J. Chem. Soc., Chem. Commun.*, 593 (1973); G. Le Corre, E. Guibe-Jampel, and M. Wakselman, *Tetrahedron*, **34**, 3105 (1978).
(31) F. G. Bordwell and A. C. Knipe, *J. Org. Chem.*, **35**, 2958 (1970); D. J. McLeunan, *Q. Rev., Chem. Soc.*, **21**, 490 (1967); J. S. Sicher, *Angew. Chem., Int. Ed. Engl.*, **11**, 200 (1972); W. T. Ford, *Acc. Chem. Res.*, **6**, 410 (1973). For peptide system, see references cited in G. Le Corre et al. (ref 30).
(32) J. M. Manning and S. Moore, *J. Biol. Chem.*, **253**, 5591 (1978).
(33) F. S. Tjoeng, J. P. Tam, and R. B. Merrifield, *Int. J. Pept. Protein Res.*, **14**, 262 (1979).

Table V. Side Reactions of Phenacyl-Resins

resin	loss after two cycles, %[a]	amino acid hydrolysis	picric acid[c] titration, %
Boc-Pro-Pro-Gly-O-$CH(CH_3)$COPh-resin (24)	35[b]	$Pro_{1.30}Gly_{1.00}$	67
Boc-Pro-Pro-Gly-OCH_2-Pop-resin (25)	<3	$Pro_{1.97}Gly_{1.00}$	98
Boc-Gly-Phe-Leu-OCH_2-Pop-resin (26)	<3	$Gly_{0.96}Leu_{0.99}Phe_{1.00}$	100
Boc-His(Tos)-Pro-Phe-OCH_2-Pop-resin (27)	<3	$His_{1.01}Pro_{0.99}Phe_{1.00}$	99

[a] Based on amino acid analysis of acid hydrolysates of the Boc amino resin and the Boc tripeptide-resin. The limit of detection is approximately 3%. [b] Taken from ref 33. [c] Reference 42, titration on the growing peptide after two cycles, % = (mmol/g of resin obtained)/(mmol/g of resin of original substitution). Also, see Experimental Section for explanation.

group. The latter is particularly dangerous because the resulting resin can be esterified by subsequent activated amino acids and start new peptide chains, which lead to truncated peptides lacking two or more residues; the former reaction is especially pronounced with C-terminal glycine, which is often present because the fragments are intended for later coupling reactions. Similar reactions have been observed by Birr.[24] Although the extent of these side reactions varies with the C-terminal residue and can be minimized with different protocols of coupling (see Experimental Section), they are only eliminated by a change in the synthetic strategy. By inserting a spacer and thereby removing the C-terminal residue from the vicinity of the ketone, the cyclization via a six-member ring cannot occur and the loss due to oxazinone formation is avoided. Furthermore, replacement of the aminoacyl phenacyl ester with an aminoacyl benzyl ester lessens the danger of diketopiperazine formation. Indeed, in a comparative study (Table V) these two side reactions did not occur within the experimental limit of detection.

Application of Multidetachable Resins to Stepwise Synthesis

To test the efficacies of the multidetachable resin, both Pop- and Pon-resins were used in stepwise synthesis to prepare the test peptide H-Leu-Ala-Gly-Val-OH[34,35] and two naturally occurring peptide hormones, Leu-enkephalin and angiotensin II. Deletion and termination peptides derived from these peptides can be identified and quantitated by ion-exchange chromatography at levels below 0.1%. The H-Leu-Ala-Gly-Val-OH obtained from solid-phase synthesis on Pop- or Pon-resins showed that the desired tetrapeptide accounted for >98.8% of the total ninhydrin-positive products present in the unpurified cleavage products. These results were comparable to those obtained with peptides synthesized on the conventional benzyl ester type of resins.

Leu-Enkephalin.[36] This simple pentapeptide was selected as a suitable test peptide to demonstrate the use of the Pop-resin. The synthesis began with the preparation of Boc-Leu-OCH_2-Pop-resin by route A, i.e., the direct esterification of Boc-Leu-OMPA with 2-bromopropionyl-resin. The chain was then extended by a standard double coupling protocol with DCC activation. The completed peptide was cleaved from the resin in four ways: (1) acidolysis with HF/10% anisole, 0.5 h, 0 °C, to give free Leu-enkephalin; (2) photolysis at >350 nm, 72 h, 25 °C, to give Boc-[Leu^5]-enkephalin-oxymethylphenylacetic acid; (3) nucleophilic cleavage by KCN/crown ether; (4) transfer hydrogenation, 18 h, 50 °C, to give Boc-[Leu^5]-enkephalin. Based on

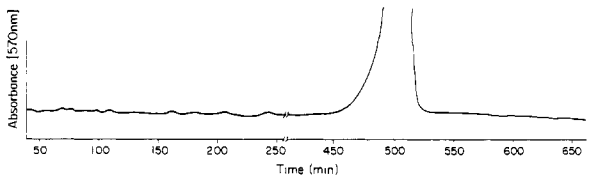


Figure 1. Ion-exchange chromatography of crude, unpurified Leu-enkephalin obtained after HF cleavage (Dowex 50 × 4, 0.9 × 55 cm, 66 mL/h, 58 °C), 0.5 M pyridine acetate (pH 5.2).

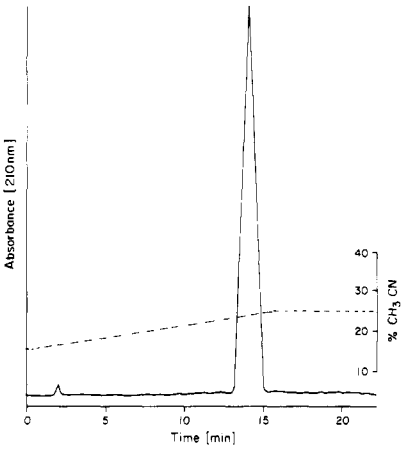


Figure 2. High-pressure liquid chromatography of crude and unpurified Leu-enkephalin obtained after HF cleavage (μBondapak C_{18} reverse phase, semipreparative column, 0.4 × 30 cm), CH_3CN and 0.1% phosphoric acid as eluent.

amino acid analysis of hydrolyzates of the protected peptide-resin and of the crude material in the filtrates, the cleavage yields were 85, 72, 76, and 55%, respectively. The two protected peptides were subsequently treated with either TFA or HF to give free Leu-enkephalin. Of the crude product obtained by direct cleavage with HF, greater than 98.5% moved as a single symmetrical peak corresponding to Leu-enkephalin in ion-exchange chromatography. The system was overloaded (5 μmol) so that side products could be detected at the 10-nmol level (>0.2%) (Figure 1). Furthermore, using C-18 reverse phase LC, the crude product appeared as a large, single peak corresponding to the authentic sample of Leu-enkephalin (Figure 2). Samples obtained by photolysis or cyanolysis followed by deprotection with HF or TFA showed similar chromatographic profiles in both systems.

Angiotensin II.[37] As a somewhat more demanding test of the multidetachable resin, the octapeptide angiotensin II was synthesized on Pop-resin. The protected Boc amino acids were Asp(OBzl), Arg(Tos), Tyr(Cl_2-Bzl), and His(Tos). C-Terminal Boc-Phe was anchored to the resin by route B (Scheme II). The synthetic protocol was otherwise the same as for the Leu-enkephalin synthesis. Aliquots of the protected peptide-resin were then cleaved in three ways: with HF, irradiation, and tetrabutylammonium cyanide to give the free angiotensin (37%), protected angiotensin-OMPA (60%), and protected angiotensin (61%), respectively. The low HF yield is attributed to an incomplete reaction of BMPA with bromo-α-methylphenacyl-resin (**11**) and therefore to the presence of significant amounts of Boc-Phe esterified directly to the resin **11**. The final peptide anchored in this way would be stable to HF, but labile to irradiation and to cyanolysis. However, of the 37% obtained by HF cleavage, 88% was found in a single chromatographic peak. We conclude that, if route B is to be used, care should be taken to assure a high yield in the above reaction. Nonetheless, the deletion of the bromomethylphenylacetic acid spacer or the presence of its dimer will not reduce the purity of the product, only its yield.

(34) R. B. Merrifield, A. R. Mitchell, and J. E. Clarke, *J. Org. Chem.*, **39**, 660 (1974).
(35) S. B. H. Kent, A. R. Mitchell, G. Barany, and R. B. Merrifield, *Anal. Chem.*, **50**, 155 (1978).
(36) J. Hughes, T. W. Smith, H. W. Kosterlitz, L. Birdsall, B. A. Morgan, and H. R. Morris, *Nature (London)*, **258**, 577 (1975); R. Simantov and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 2515 (1976).
(37) L. T. Skaggs Jr., W. H. Marsh, J. R. Kahn, and N. P. Shunway, *J. Exp. Med.*, **100**, 363 (1954); H. Schwarz, F. M. Bumpus, and I. H. Page, *J. Am. Chem. Soc.*, **79**, 5697 (1957); R. Schwyzer, B. Iselin, H. Kappeler, W. Rittel, and H. Zuber, *Chimia (Aarau)*, **11**, 335 (1957).

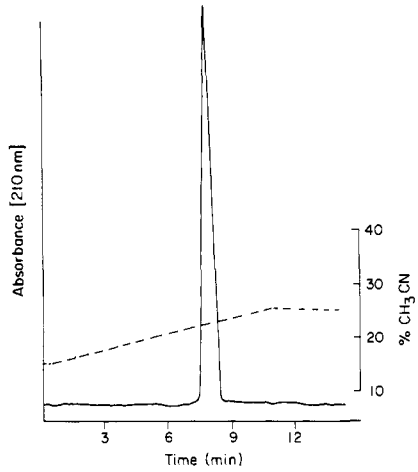


Figure 3. High-pressure liquid chromatography of purified [Val^5]angiotensin II (μBondapak C_{18} reverse phase column, 0.4 × 30 cm), CH_3CN and 0.1% phosphoric acid as eluent.

The crude product was chromatographed in a highly overloaded state on ion-exchange and C-18 reverse phase columns. In both cases the correct product was obtained in a major symmetrical peak. Of the side products, about 5% was due to the 3-(2,6-dichlorobenzyl)tyrosine-containing octapeptide resulting from rearrangement in HF. This product was isolated from both chromatographic columns and identified. The final purified product (Figure 3) obtained by ion-exchange chromatography (Dowex 50 × 4, 1 M acetate as eluent) was chemically indistinguishable by LC, TLC, and ion-exchange chromatography from an authentic, biologically active sample.

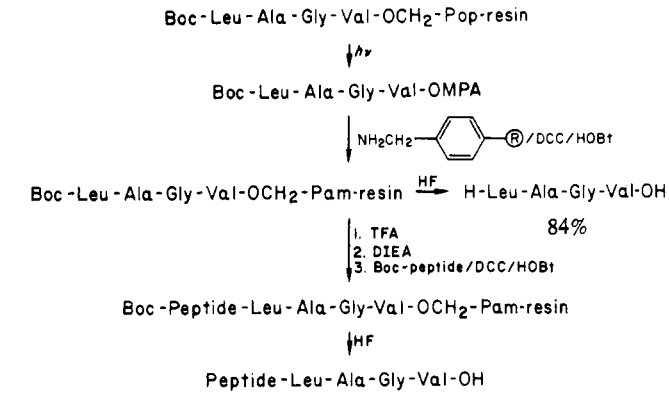
It seems clear that the Pop-resin allows a good yield of peptide of high purity to be synthesized by the standard stepwise approach and avoids several side reactions in the process. But the real value of the multidetachable resin lies in the flexibility of its application to a range of modified strategies. The product can be obtained in the free form or in two different stages of protection which can be utilized in a variety of ways.

Application of Multidetachable Resins to Fragment Synthesis

The merits of the fragment approach to the synthesis of polypeptides have been demonstrated in several notable achievements.[38-40] Such approach allows the purification and characterization of intermediates and can have advantages in the purification of the final product, but sometimes presents solubility problems in the condensation of larger fragments. One of our goals is to prepare fragments of moderate size by stepwise solid-phase synthesis, which can then be assembled on a support to form a polypeptide. This objective has been impeded by the lack of satisfactory protocols to reattach the purified peptides to the resin support. Two general methods have been applied for this purpose. First, the C-terminal amino acid or a short peptide sequence can be prepared stepwise on the usual resin support and used directly for the attachment of the next fragment. Second, the soluble C-terminal protected fragment can be purified and characterized and then attached via the cesium salt[16] to a chloromethyl-resin or bromopropionyl-resin.

The new multidetachable system offers a more satisfactory solution to the problem. The photolytic product of the Pop- or Pon-resin is the OMPA derivative of the protected peptide, which can be conveniently coupled to an aminomethyl-resin[41] to form the Boc-peptidyl-OCH_2Pam-resin. This can be further coupled with other fragments to form the larger target molecule which is finally cleaved by HF. The important points here are: first, the initial photolytic fragment is obtained in high yield because the cleavable bond is not adjacent to the C-terminal amino acid; second, this peptide is obtained in a fully protected form suitable for purification; third, the carboxyl group being activated for condensation with the aminomethyl-resin is that of the phenylacetic acid and is removed from the chiral center of the C-terminal amino acid so that steric problems are minimized and racemization does not occur.

Scheme IV. Illustration of the Use of Pop-Resin for Fragment Synthesis



To illustrate the feasibility of the system, Boc-Leu-Ala-Gly-Val-OCH_2-Pop-resin was photolyzed to give Boc-Leu-Ala-Gly-Val-OMPA, which was purified by crystallization and coupled to aminomethyl-resin with DCC to produce Boc-Leu-Ala-Gly-Val-OCH_2-Pam-resin (Scheme IV). The excess aminomethyl sites were acetylated. HF cleavage gave an 84% yield of Leu-Ala-Gly-Val which was shown to contain no D-Val diastereomer. Similarly, Boc-Leu-enkephalin-OMPA was coupled to aminomethyl-resin in 95% yield and cleaved in 82% yield by HF. These data justify the use of this approach for a fragment synthesis as illustrated in Scheme IV.

Conclusion

We believe that these multidetachable resins provide a considerable degree of freedom and versatility to the synthetic schemes that are possible. Furthermore, they are designed to avoid serious side reactions that are associated with the final HF cleavages. For example, a photolytic or cyanolytic cleavage from these resins followed by hydrogenation (or the appropriate TFA cleavage of the Boc-protecting group) will lessen the danger of alkylation of tyrosine, destruction of tryptophan, or aspartimide formation in aspartyl peptides. Moreover, the multidetachable resins also provide a route for the preparation of peptide fragments or fragments with an OMPA handle for the reattachment to the resin under mild conditions for polypeptide synthesis. The insertion of an OMPA handle between the peptide and the resin support further insulates the growing peptide from losses due to acidolytic cleavage or cyclization of the α-amino group during the synthesis as well as the enhancement of photolytic yield. We are now pursuing the application of these resins to the preparation of several large peptides to demonstrate their merit in more complex situations.

Experimental Section

High-pressure liquid chromatography (LC) was performed with two Waters Model 6000A solvent delivery systems, a Model 660 solvent programmer, and a Schoeffel variable wavelength UV detector, on μ-Bondapack-C18 column (0.4 × 30 cm). Photolysis was performed in a water-cooled photochemical reaction vessel with a photochemical immersion lamp (Ace-Hanovia, 450 W, 3-7 A, 236.54 nm) surrounded by a uranium glass filter. The solvents used for thin layer chromatography (TLC) (precoated 0.25-mm silica gel GF plates, Analtech) were 95:5 chloroform–acetic acid (CA); 85:10:5 chloroform–methanol–acetic acid (CMA), 4:1:1 1-butanol–acetic acid–water (BAW), 99:1 chloroform–methanol (CM), and 9:1 petroleum ether (bp 30–60 °C)–acetic acid

(38) H. Yajima, Y. Kiso, Y. Okada, and H. Watannke, *J. Chem. Soc., Chem. Commun.*, 106 (1974); E. Wunsch, *Angew. Chem., Int. Ed. Engl.*, **10**, 786 (1971).

(39) Protein Synthesis Group, Shanghai Institute of Biochemistry, Academia Sinica, *Sci. Sin.*, **18**, 745 (1975).

(40) N. Yanaihara, C. Yanaihara, G. Dupuis, J. Beacham, R. Camble, and K. Hofmann, *J. Am. Chem. Soc.*, **91**, 2184 (1969), K. Kawasaki, R. Camble, G. Dupuis, H. Romovacek, H. T. Storey, C. Yanaihara, and K. Hofmann, *ibid.*, **95**, 6815 (1973).

(41) A. R. Mitchell, S. B. H. Kent, B. W. Erickson, and R. B. Merrifield, *Tetrahedron Lett.*, 3795 (1976).

(PA). Spots were visualized with ultraviolet light (254 nm) or iodine vapor followed by spraying with 0.2% ninhydrin in 1-butanol and heating, with fluorescamine or with chlorine–*o*-tolidine spray. Dimethylformamide (DMF), acetonitrile, and *N*-methylpyrrolidone (NMP) were stored over molecular sieve (4 Å) for 1 week and filtered through alumina prior to use. Copoly(styrene–1%-divinylbenzene) beads, Bio Beads SX-1 (200–400 mesh), were purchased from Bio-Rad Laboratories and prewashed as follows: toluene (two times, 30 min, 70 °C), methanol (2 × 2 min), DMF (two times, 30 min, 70 °C), methanol (2 × 2 min), dioxane–2 N NaOH (1:1 v/v, 30 min, 70 °C), dioxane–H_2O (1:1 v/v, 3 × 2 min), dioxane–2 N HCl (1:1 v/v, 30 min, 70 °C), dioxane–H_2O (1:1 v/v, 3 × 2 min), methanol (3 × 2 min). The materials and methods for solid-phase synthesis were similar to those described earlier.[1,6,33]

Preparation of *tert*-Butoxycarbonylaminoacyl-4-oxymethylphenylacetic Acid (Boc-aminoacyl-OMPA, 6). General Procedure. Boc amino acid (1 mmol) was added to a suspension of potassium fluoride (anhydrous, finely pulverized under nitrogen, 3 mmol) in dimethylformamide (20 mL) at ambient temperature and 4-bromomethylphenylacetic acid phenacyl ester (0.5–0.75 mmol of BMPA-OPac) (this compound was prepared according to Mitchell et al.[6] with slight modifications to give better yield and greater purity of product;[14] KF and CH_3CN were used instead of triethylamine and ethyl acetate as base and solvent). The reaction was usually completed within 4 h, but in the case of Boc-Gly it was necessary to accelerate the reaction by heating to 50 °C. The progress of the reaction could be monitored with TLC by the disappearance of BMPA-OPac. The Boc-aminoacyl-OMPA-OPac was used after workup,[14] without further purification, for the Zn/HOAc reduction to the *tert*-butoxycarbonylaminoacyl-4-oxymethylphenylacetic acid.[6,14] All OMPA derivatives were obtained as solids except Boc-Leu-OMPA. After crystallization from EtOAc and hexane, yields were between 60 and 80%, based on the limiting reagent (BMPA-OPac). The OMPA derivatives of Boc amino acids exhibited the following spectral characteristics: NMR (acetone-d_6, ppm) 3.64 (s, 2 H, $-PhCH_2COOH$), 5.25 (s, 2 H, $-CH_2Ph-$), and 7.36 (s, 4 H, $-CH_2PhCH_2$); IR ($CHCl_3$) 1740 (carbamate), 1730 (ester), and 1720 cm^{-1} (phenylacetic acid).

Boc-Gly-OMPA (6a): mp 115–117 °C, 84% yield. Anal. Calcd for $C_{16}H_{21}NO_6$: C, 59.43; H, 6.55; N, 4.33. Found: C, 59.67; H, 6.56; N, 4.25.

Boc-Val-OMPA (6b): mp 80–83 °C, 81% yield. Anal. Calcd for $C_{19}H_{27}NO_6$: C, 62.45; H, 7.45; N, 3.83. Found: C, 62.49; H, 7.42; N, 3.80.

Boc-Leu-OMPA (6c): oil isolated as DCHA salt, mp 115–117 °C, 85% yield. Anal. Calcd for DCHA salt $C_{32}H_{52}N_2O_6$: C, 68.54; H, 9.35; N, 5.00. Found: C, 68.62; H, 9.27; N, 4.77.

Boc-Phe-OMPA (6d): mp 92–96 °C, 80% yield. Anal. Calcd for $C_{23}H_{27}NO_6$: C, 66.81; H, 6.58; N, 3.39. Found: C, 67.11; H, 6.43; N, 3.12.

Acetyl-OMPA (22): mp 99–102 °C, 79% yield. Anal. Calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found: C, 63.47; H, 5.77.

Boc-Leu-Phe-OMPA (17): mp 120–123 °C, 85% yield. Anal. Calcd for $C_{29}H_{38}N_2O_7$: C, 66.14; H, 7.27; N, 5.32. Found: C, 66.41; H, 7.13; N, 5.11.

Preparation of Resins 9–14. General Instructions. Since these resins are both photosensitive and base labile, all reactions were conducted away from light, moisture, or extensive exposure to base or nucleophile. *N*-Methylpyrrolidone or dimethylacetamide was used in place of dimethylformamide because the slow decomposition of the latter to dimethylamine, and in some cases DMF itself, led to significant side reactions with the resins during the course of esterification.[18] Esterification reactions are best conducted at ambient temperature, and heated only if required.

Preparation of 2-Bromopropionyl Resin (11).[3,18] 2-Bromopropionyl chloride (21 g, 12.3 mL, 122 mmol) was added slowly to a suspension of $AlCl_3$ (16.3 g, 122 mmol) in CH_2Cl_2 (100 mL) with gentle stirring. The brownish solution was added slowly through a dropping funnel into a 3-L three-necked round-bottom flask containing 100 g of prewashed Bio-Beads SX-1 (200–400 mesh, 1% cross-linked) in 1500 mL of CH_2Cl_2. The flask was equipped with a mechanical stirrer and condenser and maintained under positive nitrogen pressure. The mixture was stirred for 21 h. The progress of the reaction was monitored by taking a small sample of resin and observing the intensity of the 1690-cm^{-1} peak (ketone) with respect to the 1601-cm^{-1} peak (polystyrene) in the IR (KBr). When the incorporation was approximately 0.97 mmol/g of resin, the 1601/1690 ratio = 1. The acylated resin was then collected and washed successively two times each with 750 mL of CH_2Cl_2, THF, THF–anisole (9:1 v/v, to further remove acylating agents), THF, THF–H_2O (1:1, v/v), CH_3OH, DMF, and CH_3OH. The resin was off-white in color and contained orange specks of polymers, which were difficult to extract or remove under hydrolytic conditions as has been reported.[3,4] Furthermore, vigorous hydrolytic conditions would also hydrolyze phenacyl bromide resin to phenacyl alcohol-resin. In order to obtain resins free of polymeric side products, a sizing procedure was implemented here. The resin was added slowly to a 2-L graduated cylinder containing 1500 mL of CH_3OH and allowed to settle for 5 min. Supernatant, containing smaller resin beads or other polymers, was decanted. Another 1200 mL of CH_3OH was added; the resin was stirred and then allowed to settle for 5 min and the supernatant decanted. The process was repeated three times. This resin was then dried and put into a 2-L separatory funnel containing 1500 mL of CH_2Cl_2. The suspension was shaken and the resin allowed to float to the top while the debris which sank was drained off. This was repeated another four times; 63 g of resin was obtained. IR (KBr): 1690 cm^{-1}. Br (elemental 0.60 mmol/g, Volhard halide titration 0.63 mmol/g). The volume of 1.00 g of packed dry beads was 1.67 mL; after swelling in DMF, the volume was 5.3 mL, and in CH_2Cl_2 it was 7.0 mL. The 36 g of resin that was removed by the sink–float experiments was found to have 10% of Br (1.2 mmol/g). This fraction of resin could not be completely esterified by Boc amino acid.

Preparation of Bromomethyl(3-nitro)benzamidomethyl-Resin (12). This compound was synthesized as described previously by Rich and Gurwara[2] with the following modifications. The aminomethyl-resin was prepared according to the procedure described by Mitchell et al.,[41] starting from copoly(styrene–1% divinylbenzene) resin. The amount of amino sites on the resin was determined by picric acid titration[42] and found to be 0.26 mmol/g of resin. Aminomethyl-resin (18.3 g, 4.75 mmol) was added to a solution of 3-nitro-4-(bromomethyl)benzoic acid[2] (6.16 g, 23.7 mmol) and dicyclohexylcarbodiimide (4.88 g, 23.7 mmol) in 130 mL of DMF. The mixture was shaken at room temperature for 24 h and filtered. The resin was washed with DMF, CH_2Cl_2, CH_3OH, CH_2Cl_2, and DMF. Following these washes, the same amount of 3-nitro-4-(bromomethyl)benzoic acid and DCC in DMF were added to the resin and the reaction was carried out for another 18 h. After the same workup the resin was dried in vacuo. All operations were carried out in a single reaction vessel. Picric acid titration of the compound indicated that there were no free amino sites present, and elemental analysis gave 0.22 mmol Br/g of resin.

Reaction of Bromomethylphenylacetic Acid (BMPA) and Phenacyl Bromide in Potassium Fluoride. A series of experiments were carried out to optimize the conditions to prepare $BrCH_2$-Pop-resin (**13**). The experiments were designed to investigate the maximum reactivity (reaction between BMPA and phenacyl bromide) and selectivity (least amount of BMPA dimer formation). BMPA (15 mg) was allowed to react with 15 mg of KF in 0.5 mL of the following solvents: (1) DMF, (2) NMP, (3) CH_3CN, (4) toluene, (5) methylene chloride, (6) dioxane, (7) DMF–CH_3CN (1:1 v/v), (8) DMF–dioxane (1:1 v/v), (9) DMF–dioxane (1:3 v/v), (10) DMF–dioxane (1:9 v/v), (11) DMF–dioxane–CH_3CN (1:4:4 v/v), and (12) NMP–dioxane–CH_3CN (1:4:4 v/v). The disappearance of BMPA and the appearance of a new dimeric, slower moving spot were monitored by TLC (solvents CA, PA, and CMA) in each reaction. Complete disappearance of BMPA was observed within 30 min for solvents (1) and (2), 60 min for (7), 120 min for (3) and (8), 360 min for (9), but greater than 600 min for (6), (10), (11), and (12). No reaction was observed for (4) and (5) after 29 h. Using the same conditions, but with the addition of 12 mg of phenacyl bromide, formation of bromomethylphenylacetic acid phenacyl ester was observed within 30 min for (1), (2), and (7), 60 min for (3), 120 min for (8), (11), and (12), 600 min for (6), (9), and (10), and greater than 36 h for (4) and (5).[43]

Preparation of Bromomethyl-Pop-Resin (13) and Bromomethyl-Pon-Resin (14). Preliminary Experiments. A series of experiments based on the previous finding from the soluble system were set up to optimize the esterification of BMPA to the 2-bromopropionyl-resin **11**. BMPA (275 mg, 1.2 mmol) and KF (209 mg, 3.6 mmol) were added to 1 g of resin **11** (0.6 mmol) suspended in the following solvents: (1) dioxane–CH_3CN (1:1 v/v), (2) DMF–dioxane (1:9 v/v), (3) DMF–dioxane (1:3 v/v), (4) DMF–dioxane (1:1 v/v), (5) DMF–CH_3CN (1:1 v/v), (6) DMF–CH_3CN–dioxane (1:4:4 v/v), and (7) NMP–CH_3CN–dioxane (1:4:4 v/v). The reaction mixture was stirred at 50 °C for 6 h. The amount of loading was determined by IR (KBr) by comparing the carbonyl intensity of the ester peak at 1725 cm^{-1} and ketone peak at 1690 cm^{-1}. The amount of esterification was found to be 35% in solvents (1) and (2), 60% in (3) and (5), and greater than 95% in (4), (6), and (7). TLC on filtrates of these reactions indicated that BMPA still was present except for (4) and (5). When CsF was substituted for KF for experiments using solvents (1), (2), and (3), only slight improvement was noted. From these experiments it appeared that solvent (6) or (7) would be suitable to provide the maximum reactivity and selectivity.[43] Since DMF has a

(42) B. F. Gisin, *Anal. Chim. Acta*, **58**, 248 (1972).

(43) The choice of solvent is also governed by its resin-swelling property. CH_3CN does not swell the resin while dioxane does. Thus, the solvent mixture is chosen to be compatible with its swelling property, reactivity, and selectivity.

Table VI. Results of Syntheses of Boc-aminoacyl-Pop- and -Pon-Resins

from resin	Br, mmol/g	resin	route	loading of resin after esterification,[a] mmol/g Gly	Val	Leu	Phe	Leu Phe
11	(0.60)	Pop	A	0.58	0.54	0.61	0.51	0.57
12	(0.22)	Pon	A	0.21	0.19			
13	(0.42)	Pop	B	0.39	0.39	0.41	0.36	
14	(0.17)	Pon	B	0.17	0.16			

[a] Based on amino acid hydrolysis of resin in 12 N HCl-phenol-HOAc (2:1:1). Picric acid titration[44] gave good agreement (±5%) in all cases. Elemental analysis of bromine was also performed and less than 0.2% (0.025 mmol/g) was found in all cases.

tendency of decomposition to dimethylamine, solvent mixture (7) which contained NMP was used.

Larger Scale Preparation. BMPA (2.75 g, 12 mmol) and KF (2.09 g, 36 mmol) were added to a suspension of resin **11** (10 g, 6.0 mmol), or in which the molar ratio of each reactant was the same, in NMP–CH_3CN–dioxane (1:4:4 v/v). The reaction mixture was vigorously stirred for 6 h at 50 °C. The workup was the same as described earlier in the KF esterification experiments, yield 10.7 g. Anal. Br, 0.58 mmol/g. The volume of 1.00 g of packed dry beads was 1.67 mL. After swelling in dimethylformamide the volume was 5.3 mL, and in CH_2Cl_2 it was 7.0 mL. IR (KBr): 1725 (ester), 1690 cm^{-1} (ketone). Bromomethyl-Pon-resin was prepared by a similar procedure (27.5 g, 6.0 mmol). Again the yield was quantitative, and swelling properties were similar to those of bromomethyl-Pop-resin.

Preparation of *tert*-Butoxycarbonylaminoacyl-2-[4-(oxymethyl)-phenylacetoxy]propionyl-Resin (Pop-Resin, 9a–d). General Procedure (Route A). KF (anhydrous and finely ground, 3 mmol) and Boc-aminoacyl-OMPA (**6a–d**, 1 mmol) were added to a suspension of 2-bromopropionyl-resin (1 g, 0.6 mmol/g) in *N*-methylpyrrolidone (NMP, 10 mL) and vigorously mixed with an overhead paddle stirrer at ambient temperature for 24 h. Heating at 50 °C shortened the reaction time to 8 h and was necessary for more polar Boc amino acid such as Boc-Gly-OH. $KHCO_3$ (finely ground, 1 mmol) was also added after 2 h to allow the reaction to proceed to completion. However, prolonged heating often led to the undesirable result of altering the properties of these resins, which can lead to decreased yields of products. As in Boc-Gly-OCH_2-Pop-resin (**9a**), the reaction was best conducted at room temperature for 14 h followed by heating to 50 °C for an additional 4 h. The progress of the esterification was monitored by picric acid titration of small samples of resin. If necessary, reesterification with fresh OMPA derivative was carried out. The resin was then washed three times each with 15 mL of DMF, DMF–water (1:1 v/v), DMF, dioxane–acetic acid (1:1 v/v),[44] dioxane, and CH_3CN. The volume of 1.00 g of packed dry beads was 1.67 mL. After swelling in dimethylformamide the volume was 5.4 mL and in CH_2Cl_2 it was 7.1 mL.

Route B. KF (3 mmol) and Boc amino acid (1 mmol) were added to a suspension of mechanically stirred $BrCH_2$-Pop-resin (**13**, 1 g, 0.58 mmol/g) in NMP (10 mL) at ambient temperature for 36 h or at 50 °C for 12 h. Esterification by the Cs salt method according to Gisin could also be used,[16] but some discoloration of the resin was observed using this procedure. The workup was the same as in route A. Results of these preparations are tabulated in Table VI.

Preparation of *tert*-Butoxycarbonylaminoacyl-4-[4-(oxymethyl)-phenylacetoxymethyl]-3-nitrobenzamidomethyl-Resin (10a,b, Pon-Resin). General Procedure (Route A). KF (0.90 mmol) and Boc-aminoacyl-OMPA (**6a,b**, 0.3 mmol) were added to a suspension of mechanically stirred 4-bromomethyl-(3-nitro)benzamidomethyl-resin (**12**, 1 g, 0.22 mmol/g) in *N*-methylpyrrolidone (10 mL) at ambient temperature for 18 h. Workup was the same as described for the Pop-resin (**9**). Results are tabulated in Table VI.

Route B. KF (0.75 mmol) and Boc amino acid (0.25 mmol) were added to a suspension of mechanically stirred $BrCH_2$-Pon-resin (1 g, 0.15 mmol/g) in NMP (10 mL) at ambient temperature for 30 h. Workup was the same as described for the Pop-resin (**9**). Results are tabulated in Table VI.

Methods for Cleavage of Boc-aminoacyl-OCH_2-Pop- or -Pon-Resins. A. HF. Two mixtures consisting of 100-mg samples of **9a–f** and 100-mg samples of **10a,b** were treated with 9 mL of HF and 1 mL of anisole at 0 °C for ~0.5 h. After removal of HF in vacuo at 0 °C, the resin was washed three times with 4 mL of cold ethyl acetate and extracted five times with 4 mL of 50% acetic acid. The combined acetic acid layer was then diluted to 40 mL and lyophilized. The resulting amino acids were dissolved in citrate buffer with norleucine standard for analysis. The results are shown in Table II.

B. Methanesulfonic Acid. Two mixtures consisting of 100-mg samples of **9a–d** and 100-mg samples of **9e,f** were treated with 20 mL of MSA–TFA–anisole–CH_2Cl_2 (5:2:1:2 v/v) at 24 °C for 1 h. After removal of CH_2Cl_2 and TFA the resulting oily residue was diluted with 100 mL of water and adjusted to pH 7 with NaOH. An aliquot was taken out and combined with norleucine as internal standard in citrate buffer for amino acid analysis. The results are shown in Table II.

C. Photolysis. Samples (100 mg) of each preparation, **9a–f** and **10a,b**, were suspended in 10 mL of DMF in screw-capped Pyrex test tubes. The samples were degassed for 1 h in a steady stream of nitrogen and then photolyzed while stirring at about 6 in. from the Hanovia light source for 72 h. In each case the resin was filtered and washed three times with 5 mL of DMF. The remaining resin was dried and hydrolyzed in 2 mL of HCl–HOAc–88% PhOH (2:1:1 v/v) for 24 h at 120 °C. The value for % cleavage calculated from this hydrolysis was averaged with that calculated from the 6 N HCl hydrolysis of an aliquot of the DMF filtrate (Table IV). To identify the product as Boc-aminoacyl-OMPA, aliquots were compared with authentic OMPA derivatives by TLC. Only OMPA derivatives were obtained. Aliquots of the DMF filtrates were evaporated, deprotected in TFA, and examined by quantitative amino acid analysis. In each case less that 0.5% of free amino acid was present. Another aliquot of each DMF filtrate was deprotected by catalytic hydrogenolysis over 5% $Pd/BaSO_4$ in 95% ethanol. The products were shown by TLC to be identical with authentic samples of the corresponding Boc amino acids and peptides.

D. Cyanolysis. Samples (100 mg) of each preparation, **9a–d**, were treated with 1 mL of 1 M tetrabutylammonium cyanide (waxy-white solid, recrystallized from EtOAc) or 1 M KCN/crown ether (1:1 complex) in DMF. The samples were stirred for 2–4 h in the dark. The resins were filtered and washed with 3 × 2 mL of DMF. The combined DMF filtrates were adjusted in the hood to pH 7 (HCN!, caution) with dilute HCl, evaporated to dryness, and extracted into EtOAc. Aliquots were deprotected with TFA for amino acid analysis and compared with authentic Boc amino acid in TLC. The cleaved resin was dried, hydrolyzed, and analyzed for residual amino acids (Table IV).

E. Aqueous Saponification. Two mixtures consisting of 100-mg samples of **9a–d** and 100-mg samples of **9e,f** were stirred in 4 mL of Triton B–water–dioxane (1:3:6). Since Triton B is 40% hydroxide ion in CH_3OH, the actual concentration of ^-OH is calculated to be 0.25 N. Each reaction was allowed to proceed for 2 h at ambient temperature. After filtration, washing, and acidification, only Boc amino acids of **9a–d** and **10a,b** were found. The cleavage yields are tabulated in Table IV.

F. Thiolysis. Thiophenoxide was generated in situ by the addition of 110 mg of thiophenol (final concentration 1 M) to 1 mL of a thoroughly degassed 1 M solution of triethylamine in DMF. Then 100-mg samples of **9a–d** were added and the reactions were each allowed to proceed for 24 h (Boc-Val-OCH_2-Pop-resin (**9b**) required 36 h). Boc-aminoacyl-OMPA **6a–d** was the only observed product for each reaction. Reaction yields were based on the hydrolysis of the resulting resin and are tabulated in Table IV.

G. Hydrazinolysis. Separate 100-mg samples of **9a–d** and **10a,b** were allowed to react at room temperature with 4 mL of 10% hydrazine in DMF for 1 h. The Boc-aminoacyl hydrazides of **10a,b** were identical with authentic samples. Yields shown in Table IV were based on acid hydrolysis of the remaining resin.

H. Hydrogenolysis. (a). Two mixtures of 100-mg samples each of **9a–d** and, in a separate experiment, 100-mg samples each of **10a,b** were treated with 4 mL of DMF (3 mL for **10a,b**) and 896 mg of palladium(II) acetate (4 mmol). Each reddish slurry was shaken slowly in a heated Parr-shaker hydrogenation apparatus at 50 °C followed by the introduction of hydrogen gas. The solution turned clear with the resin coated as a large "coal ball" with the Pd black and was allowed to shake for 17 h at 50 °C. The resin was then filtered and washed three times with 4 mL of DMF. The solution was evaporated to dryness, deprotected by 20 mL of TFA for 0.5 h, dried, and quantitated using the Beckman amino acid analyzer 121. Results are shown in Table IV.

(b). Two mixtures of 100-mg samples each of **9a–d** and, in a separate experiment, 100-mg samples of **10a,b** were treated with 4 mL of DMF and 896 mg of palladium(II) acetate (4 mmol) for 15 min in an oil bath at 50 °C. 1,4-Cyclohexadiene (0.2 mL) was then added and the reaction mixture was stirred by a gentle stream of nitrogen gas for 17 h. Three more additions of cyclohexadiene were made at 3-h intervals. The workup was similar to that described above. Results are shown in Table IV.

Preliminary hydrogenation results using a low concentration of palladium(II) acetate (4 molar equiv compared to the resin) led to erratic

(44) Acetic acid–dioxane mixture (1:1) was found to be most effective to completely remove all inorganic fluoride ion.

results and lower yields. Both hydrogenation methods provided only the corresponding Boc amino acids from resins **9a–d** and **10a,b**. No OMPA derivatives were observed by TLC when compared with the authentic sample.

(c). Hydrogenation of Peptide-Resins. Boc-[Leu^5]-enkephalin-OCH_2-Pop-resin (100 mg, 0.61 mmol/g) was treated as in B with 224 mg of palladium(II) acetate (1 mmol) in 1.2 mL of DMF at 50 °C for 17 h to obtain, after workup and TFA deprotection, 13.5 mg (55%) of [Leu^5]enkephalin. Boc-Leu-Ala-Gly-Val-OCH_2-Pon-resin (100 mg, 0.16 mmol/g) was treated similarly to give 3.7 mg (61%) of Leu-Ala-Gly-Val. Both products were found to be identical with the authentic samples in their behavior on ion-exchange columns.

Stability of Boc-aminoacyl-OCH_2-Pop-Resins (9a–d) in Refluxing Trifluoroacetic Acid. Samples of Boc-aminoacyl-OCH_2-Pop-resin (**9a–d**) (100 mg each), prepared by route A, were placed in 25-mL round-bottom flasks equipped with a water condenser and drying tube. Anhydrous trifluoroacetic acid (10 mL) was added and the stirred suspensions were heated to reflux in a preheated oil bath at 135 °C. After periods of 2, 4, 6, 8, 10, and 12 h samples were filtered and washed with trifluoroacetic acid. The filtrates were evaporated, dissolved in water, and analyzed for amino acid content. The results are summarized in Table III.

Treatment of Boc-aminoacyl-OCH_2-Pop-Resins (9a–d) with Amines. A. Reaction with Benzylamine. Samples of Boc-aminoacyl-OCH_2-Pop-resin (100 mg each) were suspended in 10 mL of 1 M benzylamine–dichloromethane for 1 h. The suspensions were then filtered and washed. The residual resin was hydrolyzed and the following results based on amino acid analysis were obtained (in percentage as compared to the original substituion): Gly-**9a**, 97.2; Val-**9b**, 98.8; Leu-**9c**, 99.1; Phe-**9d**, 99.2.

B. Reaction with Triethylamine. Samples of **9a–d** (100 mg each) were treated with 5% triethylamine–dichloromethane (v/v) for 24 h. The workup and analyses were similar to those described above. The following results (in percentage as compared to the original substitution) were obtained: **9a**, 99.1; **9b**, 99.6; **9c**, 99.5; **9d**, 99.6. Analysis of the filtrate agreed with these results.

After the completion of the synthesis of Leu-enkephalin on resin **9c**, the peptide resin was hydrolyzed to determine the loss of peptide after four sequential base-neutralization steps. The peptide content based on Phe was found to be 99.0% as compared to the original substitution of Leu loading on the resin **9c**.

Test for Racemization.[32,45] Samples of Boc-Leu-Phe-OCH_2-Pop-resin[18] (100 mg, 0.57 mmol/g) prepared by esterifying Boc-Leu-Phe-OMPA to resin **11** were cleaved by HF, 1 M KCN/crown ether, and photolysis. All cleavages followed the procedure described in the methods section. Samples after cyanolytic cleavage were further treated with 50% TFA–CH_2Cl_2 for 0.5 h to remove the Boc group. The photolytic sample was hydrogenolyzed over 5% Pd/$BaSO_4$ to remove the oxymethylphenylacetic acid moiety, followed by acidolyic removal of the Boc group. All samples were then dissolved in 5 mL of water and 1-mL samples were chromatographed on the long column of the amino acid analyzer (0.9 × 58 cm, Beckman AA-15 sultonated polystyrene) in pH 5.26 buffer (Beckman citrate buffers). L-Leu-L-Phe eluted at 87 min and L-Leu-D-Phe at 104 min. Only the L-L diastereomer was detected, indicating the presence of less than 0.1% of the L-D isomer and therefore no measurable racemization.

Attempts to Detect Loss of Peptide after Two Cycles of Synthesis Using Pop-Resin.[33] The method to detect the loss of peptide chain during the first two cycles of synthesis was performed as reported in our earlier work on the synthesis of an immunoglobulin fragment on the phenacyl ester type resin.[33] Boc-Pro-Pro-Gly-resin (**24**) was synthesized from Boc-Gly-O-2-propionyl-resin (200 mg, 0.42 mmol/g) as described in ref 33. Boc-Pro-Pro-Gly-OCH_2-Pop-resin (**25**) was synthesized stepwise according to the described procedure in the earlier section from Boc-Gly-OCH_2-Pop-resin (**9a**, 200 mg, 0.58 mmol/g). Boc-Gly-Phe-Leu-OCH_2-Pop-resin (**26**) and Boc-His(Tos)-Pro-Phe-OCH_2-Pop-resin (**27**) were the C-terminal three residues of Leu-enkephalin and angiotensin II syntheses as described in the later section; 200 mg of **27** or **28** was used for the analysis. Picric acid analysis on Pro-Gly resin gave 0.28 mmol/g of growing peptide chain, a 33% loss. Amino acid hydrolysis of Pro-Pro-Gly-resin 24 gave Pro, 1.30; Gly, 1.0. Picric acid analysis gave 0.25 mmol/g of amino group, indicating a 40% loss due to termination or cyclization of Pro-Gly. Similar analyses on samples **25**, **26**, and **27** showed negligible loss within the detection limit due to termination or deletion in the first two cycles. Results are shown in Table V.

Application of Multidetachable Resins to Stepwise Synthesis. General Synthetic Protocol. Because of the base and nucleophilic labilities of these resins, a modified synthetic protocol was adopted with shortened neutralization and coupling times, a mixed-mode coupling, and the use of CH_3CN as shrinking solvent. The complete protocol is shown in Table VII.

Table VII

50% TFA–CH_2Cl_2[a]	1 × 2 min
50% TFA–CH_2Cl_2	1 × 20 min
CH_2Cl_2	5 × 1 min
5% DIEA–CH_2Cl_2[b]	3 × 1 min
CH_2Cl_2	3 × 1 min
preformed Boc amino acid symmetric anhydride (3 equiv in CH_2Cl_2)	1 × 30 min
CH_2Cl_2	5 × 1 min
5% DIEA–CH_2Cl_2	3 × 1 min
CH_2Cl_2	5 × 1 min
Boc amino acid (3 equiv in DMF)[c] ⩽ DCC–HOBt[d] (3 equiv each)	1 × 2 min 1 × 30 min
CH_2Cl_2	3 × 1 min
CH_3CN	3 × 1 min
CH_2Cl_2	3 × 1 min
	total 115 min

[a] TFA = trifluoroacetic acid. [b] DIEA = diisopropylethylamine. [c] Do not filter. [d] DCC = dicyclohexylcarbodiimide; HOBt = 1-hydroxybenzotriazole. A terminal program is used after the incorporation of the last amino acid, HOAc–dioxane (1:1 v/v, 2 × 2 min), dioxane (2 × 2 min), CH_3CN (3 × 1 min).

A. H-Leu-Ala-Gly-Val-OH. Two syntheses were performed. One was on Boc-Val-OCH_2-Pop-resin (**9b**, 500 mg, 0.54 mmol/g, prepared by route A) and the other was on Boc-Val-OCH_2-Pon-resin (**9f**, 300 mg, 0.16 mmol/g, prepared by route B). The syntheses were performed according to the above protocol. Aliquots of each tetrapeptide-resin were cleaved by several methods and the results are tabulated in Tables II and III. To test the efficacies of the resins, the crude peptide obtained after HF cleavage was dissolved in 2–4 mL of water and 1 mL was injected into the AA-15 cation exchange resin column (0.9 × 58 cm) on a Beckman 120B amino acid analyzer and eluted with pH 3.49 citrate buffer (66 mL/h, 58 °C). The sample was intentionally overloaded (5–7 μmol) so that less than 0.1% of ninhydrin-positive components could be quantitated. Leu-Ala-Gly-Val, obtained from resin **9b**, showed a major peak of LAGV at 234 min (98.8 mol %) and <0.05 mol % of any deletion peptide. Leu-Ala-Gly-Val obtained from **10b** also showed the desired tetrapeptide (98.9 mol %) and <0.05 mol % of any deletion peptide. No diastereomers of the tetrapeptide were detected in either case.[34,35,45]

B. Leu-Enkephalin. The peptide was synthesized from Boc-Leu-OCH_2-Pop-resin (**9c**, 3 g, 0.61 mmol/g, prepared by route A); Boc-Tyr-OH was unprotected on the side chain. During the synthesis, small portions of resins were retrieved at each step for picric acid titration and amino acid analysis. Both analyses indicated that there was no significant termination or deletion in the synthesis. Cleavage of resins was performed on 100–250-mg samples with the following yields: 85 (HF), 72 (photolysis), and 76% (KCN/crown ether). The unpurified peptide sample obtained from HF cleavage (10 mg) was chromatographed on a Dowex 50 column (0.9 × 55 cm, 66 mL/h, 58 °C) of a Beckman 120B amino acid analyzer in 0.5 M pyridine acetate, pH 5.2. The major peak at 285 min as shown in Figure 1 contained 98.5 mol % of the total ninhydrin-positive material. Analysis of the unpurified material by LC (Figure 2) on a C-18 reverse phase column (0.4 × 30 cm) with a CH_3CN–0.1% phosphoric acid gradient showed a large, symmetrical peak at 8.0 min, with only traces of base-line noise. In both cases, the chromatographic behavior of the sample was identical with that of the authentic sample obtained from other sources. Amino acid analysis: Gly, 2.00; Leu, 1.01; Phe, 1.01; Tyr, 0.98.

C. [Val^5]Angiotensin II. The peptide Boc-Asp(OBzl)-Arg(Tos)-Val-Tyr[2,6-Cl_2Bzl)-Val-His[Tos]-Pro-Phe-OCH_2-Pop-resin was synthesized from Boc-Phe-OCH_2-Pop-resin (**9d**, 3 g, 0.36 mmol/g, prepared by route B) according to the protocol described earlier. Samples of 200–500 mg each were cleaved by HF, 1 M tetrabutylammonium cyanide, and photolysis. HF cleavage of 500 mg of the peptide-resin gave 49 mg (37% yield) of crude peptide. When 5 mg of the crude peptide was applied to a Beckman sulfonated polystyrene Dowex 50 × 4 column (0.9 × 58 cm) and eluted with 1 M pyridine–acetate buffer at pH 5.2, the major peak corresponding to [Val^5]angiotensin at 309 min contained 87.5% of all ninhydrin-positive materials. Three other peaks (188, 236, and 292 min) accounted for 11.2%, of which 4.6% corresponded to the rearranged alkylated [3-(2,6-Cl_2Bzl)Tyr^4]angiotensin side product. A larger sample (35 mg) was purified by preparative chromatography on a Dowex 50 × 4 column (0.9 × 58 cm). Homogeneous material (25 mg) was obtained from the peak corresponding to angiotensin. Amino acid analysis: Asp, 1.03; Val, 1.96; Phe, 1.00; Tyr, 0.97; His, 0.99; Arg, 1.00;

(45) A. R. Mitchell, S. B. H. Kent, I. C. Chu, and R. B. Merrifield, *Anal. Chem.*, **50**, 637 (1978).

Pro, 1.01. The crude product when rechromatographed on a PA-35 column or on a C-18 reverse phase column eluted with CH_3CN–0.1% phosphoric acid gradient (0.4 × 30 cm) in LC showed a symmetrical peak (Figure 3). This product also eluted as a single peak in both systems when cochromatographed with standard, biologically active [Val^5]angiotensin II.

Cyanide and photolytic cleavage gave 60 and 61% yields, respectively. When both products were further deprotected in HF and applied to the Dowex 50 × 4 ion-exchange column, each showed a major peak at 309 min (86.2 and 89.5% of ninhydrin-positive materials) corresponding to angiotensin. The photolytic cleavage product gave a mixture of about 2:1 of angiotensinyl-OMPA and protected angiotensin, indicating an incomplete conversion of resin **11** to $BrCH_2$-Pop-resin before the esterification step of Boc-Phe. This would account also for the low yield of the HF cleavage. All reactions were not optimized.

Reattachment of Boc-Leu-Ala-Gly-Val-OMPA and Boc-[Leu^5]Enkephalin-OMPA to Aminomethyl-Resin. Boc-Leu-Ala-Gly-Val-OMPA and Boc-[Leu^5]enkephalin-OMPA were each obtained from the photolysis of 200-mg resin samples of Boc-Leu-Ala-Gly-Val-OCH_2-Pop-resin and Boc-[Leu^5]enkephalin-OCH_2-Pop-resin. After the evaporation of the photolysate DMF filtrate both samples were used for the reattachment experiments. After a single precipitation from EtOAc–hexane as slightly yellowish waxy solids, both samples gave one major ninhydrin-positive spot on TLC (CMA, CA). Boc-Leu-Ala-Gly-Val-OMPA (20 mg, 33 μmol) was activated by DCC (10 mg, 48 μmol) and HOBt (7 mg, 48 μmol) at 0 °C in 2 mL of CH_2Cl_2/DMF (1:1 v/v) for 1 h and followed by addition of aminomethyl resin[41] (200 mg, 0.25 mmol/g). The coupling was conducted for 1 day. The resin was then washed five times each with 5 mL of DMF and CH_3CN, dried, and analyzed as follows.

(1) Amino acid analysis (25 mg) gave 81% coupling yield based on Leu ($Leu_{1.00}Ala_{0.95}Gly_{1.07}Val_{0.98}$).

(2) HF cleavage (70 mg) gave 84% yield of LAGV based on the chromatographic analysis of the product on a Beckman 120B[45] and the amino acid analysis of the resin. Similarly, Boc-[Leu^5]enkephalin-OMPA (18 mg, 22 μmol) was coupled to aminomethyl-resin (200 mg, 0.25 mmol/g). Amino acid analysis (28 mg of resin) gave 95% coupling yield based on Tyr ($Tyr_{1.00}Gly_{2.18}Phe_{0.98}Leu_{0.95}$) and 82% yield based on amino acid analysis of the resulting resin, HF cleavage Boc-[Leu^5]-enkephalin-OCH_2-Pam-resin.

Acknowledgments. We wish to thank Drs. S. B. H. Kent and S. Wolff for helpful discussions during the course of this work. We also thank Ms. N. Wu and A. McNichol for technical assistance, and Ms. M. LeDoux for the amino acid analyses. This work was supported in part by Grant AM 01260 from the U.S. Public Health Service and by a grant from the Hoffmann-La Roche Foundation.

2,5-Diphenyl-2,5-norbornyl Dications[1]

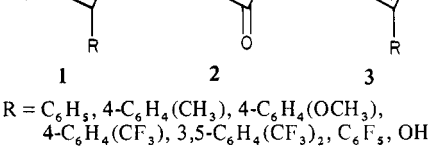
George A. Olah,* G. K. Surya Prakash, and Tarik N. Rawdah

Contribution from the Hydrocarbon Research Institute and Department of Chemistry, University of Southern California, Los Angeles, California 90007. *Received March 18, 1980*

Abstract: Preparation and ^{13}C NMR spectroscopic study of the parent and a series of substituted 2,5-diphenyl-2,5-norbornyl dications **1**-R with both electron-releasing and electron-withdrawing substituents were carried out to determine the effect of dipositive charge on the norbornyl skeleton. A plot of C-1 vs. C-3 carbon chemical shifts shows an excellent linear fit with a wide variety of substituents, indicating the regular phenylcarbenium ion nature of dications **1**-R in contrast to 2-phenyl-2-norbornyl monocations **3**-R, where significant deviation from linearity was observed in the case of electron-withdrawing substituents due to the onset of nonclassical σ delocalization. The observed behavior of dications can be rationalized by charge–charge repulsion resulting in increased charge delocalization into the phenyl rings.

Introduction

Experimental and theoretical studies on carbodications are extremely sparse as compared to those on carbomonocations.[2] In the early 1960s the first carbodications were reported by Hart[3] and subsequently by Volz.[4] In these studies the two carbocation centers were stabilized by conjugation with aromatic rings. The first aliphatic carbodications were reported by Olah et al.,[5] who showed that these ions can be formed only if the carbocation centers are separated by at least two carbon atoms. Since then a limited number of carbodications such as Hückeloid cyclic dications,[6] including cyclobutadiene and cyclooctatetraene dications, Hogeveen's pyramidal dication,[7] rigid bridgehead bicyclo[2.2.2]octyl[8] and bicyclo[3.3.3]undecyl[9] dications, and alkyl/aryl substituted acyclic dications,[10] have been studied. In our continuing studies on carbodications we now report the preparation and ^{13}C NMR spectroscopic study of the parent and a series of substituted 2,5-diphenyl-2,5-norbornyl dications (**1**-R).



1 **2** **3**

R = C_6H_5, 4-$C_6H_4(CH_3)$, 4-$C_6H_4(OCH_3)$, 4-$C_6H_4(CF_3)$, 3,5-$C_6H_4(CF_3)_2$, C_6F_5, OH

Introduction of two electron-deficient centers into the norbornyl skeleton and the effect of substituted phenyl rings on the system are of particular interest in view of the extensive studies[11] on the

(1) Stable Carbocations 230. For part 229, see: Olah, G. A.; Prakash, G. K. S.; Nakajima, T., *Angew. Chem.*, in press.

(2) For reviews see: (a) Olah, G. A.; Pittman, Jr.; Symons, M. C. R. In "Carbonium Ions", Olah, G. A., Schleyer P. v. R., Eds.; Wiley-Interscience: New York, Vol. 1, 1969; pp 135–151. (b) Olah, G. A. "Carbocations and Electrophilic Reactions"; Verlag Chemie, Wiley-Interscience: New York, 1974; pp 15–29. (c) Olah, G. A. *Top. Curr. Chem.* **1979**, *80*, 21–88.

(3) Hart, H.; Sulzberg, T.; Rajos, R. R. *J. Am. Chem. Soc.* **1963**, *85*, 1800–1806.

(4) Volz, H.; Volz de Lecia, H. J. *Tetrahedron Lett.* **1964**, 1871–1874.

(5) Bollinger, J. M.; Cupas, C. A.; Friday, K. J.; Wolfe, M. L.; Olah, G. A. *J. Am. Chem. Soc.* **1967**, *89*, 156–158.

(6) (a) Paquette, L. A. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 106–117. (b) Olah, G. A.; Staral, J. S. *J. Am. Chem. Soc* **1976**, *98*, 6290–6304. (c) Olah, G. A.; Staral, J. S.; Liang, G.; Paquette, L. A.; Melega, W. P.; Carmody, H. J. *Ibid.* **1977**, *99*, 3349–3355.

(7) Hogeveen, H.; Kwant, P. W. *J. Am. Chem. Soc.* **1974**, *96*, 2208–2214.

(8) Olah, G. A.; Liang, G.; Schleyer, P. v. R.; Engler, E. M.; Dewar, M. J. S.; Bingham, R. C. *J. Am. Chem. Soc.* **1973**, *95*, 6829–6831.

(9) Olah, G. A.; Liang, G.; Schleyer, P. v. R.; Parker, W.; Watt, C. I. F. *J. Am. Chem. Soc.* **1977**, *99*, 966–968.

(10) Olah, G. A.; Grant, J. L.; Spear, R. J.; Bollinger, J. M.; Serianz, A.; Sipos, G. *J. Am. Chem. Soc.* **1975**, *97*, 2501–2507.

(11) (a) Olah, G. A. *Acc. Chem. Res.* **1976**, *9*, 41–52. (b) Brown, H. C.; "The Non-Classical Ion Problem"; Plenum Press: New York, 1977.

0002-7863/80/1502-6127$01.00/0