From these relationships, the standard deviation in the calculated C–C bond lengths is reduced to only ~0.01 Å, which is comparable to the accuracy of the measurements. The 4-31G geometries are closely related to the 6-31G* structures (slope 0.99), but the 3-21G geometries are slightly different (slope 0.95).

Calculations. The calculations were carried out by the program GAMESS.[22] Except for the two cases noted in the table, all energies in Table III correspond to structures that have been optimized with the same basis set. The times required for the computation of the gradient of the energy with respect to the coordinates, which is the most time-consuming part of the calculations, are given in Table V for a VAX-11/750 with a floating point accelerator. The differences in times for compounds with the same number of atoms results from the differences in symmetry.

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A Gradative Deprotection Strategy for the Solid-Phase Synthesis of Peptide Amides Using *p*-(Acyloxy)benzhydrylamine Resin and the S_N2 Deprotection Method

James P. Tam

The Rockefeller University, New York, New York 10021

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An efficient deprotection strategy for the preparation of peptide amides by solid-phase peptide synthesis is described. The new method, gradative deprotection approach, utilized a multidetachable benzhydrylamine resin, *p*-(acyloxy)benzhydrylamine resin, and a mild S_N2 deprotection method for the removal of benzyl protecting groups. The multidetachable resin was designed to contain dual properties. The weakly electron-withdrawing *p*-acyloxy substituent on the benzhydrylamine linkage to the resin provided the required acid stability for the repetitive CF_3CO_2H treatments during synthesis and the S_N2 deprotection of all benzyl protecting groups after the completion of the synthesis. Under such a treatment, the crude and deprotected peptide remained attached on the resin support. Liberation of the peptide from the resin support by a nucleophile also concomitantly converted the *p*-acyloxy moiety to a strongly electron-donating *p*-hydroxy substituent on the benzhydrylamine, which would be smoothly removed by a mild acidic solvolytic treatment to give the peptide amide. Thus, the gradative deprotection approach consisted of multisteps and deprotected peptides from the resin support in discrete and controlled conditions to minimize strong-acid-catalyzed side reactions. Pentagastrin, H-Gly-Trp-Met-Asp-Phe-NH_2, was obtained in 90% overall yield and greater than 98% purity when deprotected by this new approach.

The conventional strategy in the chemical synthesis of peptides[1] by the solid-phase method[2] usually adopts a final, one-step, S_N1 cleavage process to remove protecting groups and the resin support by a very strong acid. It is now known that many protecting groups and side chain functionalities of the peptide product would best be removed under a milder condition to avoid the consequence of generating several serious side reactions.[3] A practical approach to this problem is to adopt a gradative process of deprotection that is discretely gradual and controlled.[4,5] Such an approach will deprotect the synthetic peptide after the completion of the synthesis in a stepwise fashion with the minimal required strength of acidity at each step and, thus, will likely avoid many of the known side reactions catalyzed by strong acids.

In essence, the gradative deprotection method is a multistep deprotection process after the completion of the peptide synthesis to produce peptide amides[6-10] using the conventional combination of N^{α}-(*tert*-butyloxy)carbonyl and benzyl side chain protection groups on a modified benzhydrylamine support, *p*-(acyloxy)benzhydrylamine[5] (Figure 1). The deprotection is carried out in four steps: First, the N^{α}-(*tert*-butyloxy)carbonyl group is removed by trifluoroacetic acid to eliminate the *tert*-butyl cationic source, which may lead to alkylation side reactions.[11] Second, the benzyl protecting groups are removed by a mild S_N2 deprotection method[5] with the crude and free peptide still attached to the resin after this treatment.

(1) Abbreviations follow the tentative rules of the IUPAC–IUB commission on Biochemical Nomenclature, published in: *J. Biol. Chem.* **1972**, *247*, 979–982. Others: ABA, *p*-(acyloxy)benzhydrylamine; Boc, (*tert*-butyloxy)carbonyl; DCC, dicyclohexylcarbodiimide; DIEA, diisopropylethylamine; DMAP, (*N,N*-dimethylamino)pyridine; HOBt, 1-hydroxybenzotriazole; TEA, triethylamine; TFA, trifluoroacetic acid; TFMSA, trifluoromethanesulfonic acid.

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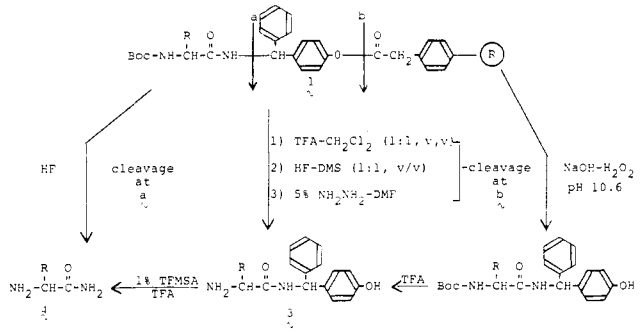

Figure 1. Design of the multidetachable *p*-(acyloxy)-benzhydrylamine resin.

Third, the deprotected peptide, together with its benzhydrylamine handle, is released from the resin support by a nucleophile. Last, the benzhydrylamine handle is cleaved by solvolysis in a dilute solution of a strong acid to give the desired peptide amide. In this paper, the gradative deprotection method, the preparation, and the properties of the *p*-(acyloxy)benzhydrylamine are described.

Results

Rationale and Design of the *p*-(Acyloxy)-benzhydrylamine Resin. The design of the new resin utilizes the different electronic effects transmitted through the 1,4-disubstituted phenyl ring of the benzhydryl moiety to modulate the acid stabilities of the benzhydrylamine bond. Electron-donating *p*-hydroxy or *p*-alkoxy substituents on a benzhydrylamine linkage will generally decrease its acid stability, particularly toward trifluoroacetic acid. Direct utilization of such an activating substituent on the benzhydrylamine linkage will not be compatible with the present synthetic strategy in which N^{α}-(*tert*-butyloxy)-carbonyl and side-chain benzyl protection groups are used. However, an electron-withdrawing substituent on benzhydrylamine linkage will provide a deactivating effect and increased acid stability. Thus, by manipulating the electronic effect on the benzhydrylamine substituent, one could produce the desired effect and result based on the differential acid stability. The strategy adopted for the present multidetachable benzhydrylamine employs a masked form of the weakly deactivating *p*-(acyloxy)-benzhydrylamine **1** (Figure 1) linkage to give it stability to repetitive trifluoroacetic acid treatments. Since the acyloxy linkage is also a phenyl ester bond, treatment of the *p*-(acyloxy)benzhydrylamine (ABA) resin with a nucleophile or base (e.g., NH_2NH_2) will free the peptide as the *p*-hydroxybenzhydrylamine **3** (Figure 1) from the resin support. The *p*-hydroxy substitution on the benzhydrylamine bond becomes strongly electron donating and causes this linkage to be more acid labile and removable by a dilute rather than a concentrated solution of strong acid to give the desired peptide **4**. Such a design will allow other synthetic schemes to obtain a protected peptide fragment containing a removable handle **2** capable of being reattached to the carboxymethyl resin.

Preparation of *p*-(Acyloxy)benzhydrylamine Resin. The synthesis of the *p*-(acyloxy)benzhydrylamine resin support has two aspects: a convenient preparation of the removable handle *N*-Boc-*p*-hydroxyybenzhydrylamine (**7**) and an efficient method of attachment to the appropriate functionalized resin support.

A. Synthesis of the Removable Handle. *N*-Boc-*p*-hydroxybenzhydrylamine (**7**) was prepared in three steps. Condensation of *p*-hydroxybenzophenone under the Leukart condition[13,14] gave *N*-formyl-*p*-hydroxybenzhydrylamine (**5**) in excellent yield (Figure 2). Since the expected electron-donating *p*-hydroxy substitution on the benzhydryl moiety of **5** greatly increased the susceptibility to deamination in the acid treatment, the hydrolytic removal of the *N*-formyl group was therefore carried out under a controlled and milder condition of 2 N HCl at 75 °C for 0.8 h instead of the normal recommended condition[6] of 6 N HCl for 4 h at 110 °C.

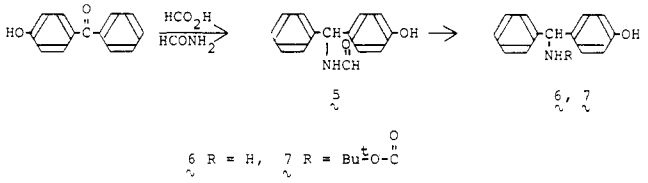

Figure 2. Preparation of *N*-Boc-*p*-hydroxybenzhydrylamine.

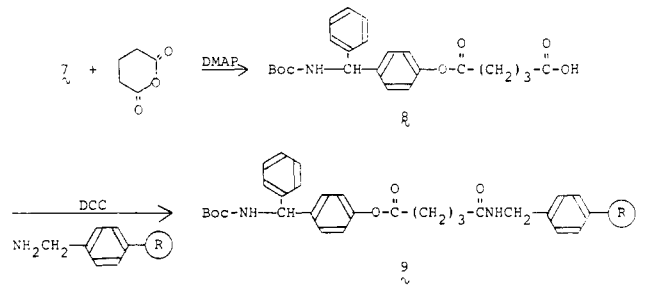

Figure 3. Attachment of *N*-Boc-*p*-hydroxybenzhydrylamine to carboxymethyl resins.

The *N*-formyl protecting group is known to be resistant to base, and the conversion of **5** to **6** by alkali hydrolysis was not successful. However, treatment of **5** with hydrazine acetate in ethanolic solution at 45 °C for 36 h did lead to the removal of the *N*-formyl group[15] and provided an alternative route to its synthesis. Synthesis of **6** using the oxime method has also been reported.[9]

N-Boc-*p*-hydroxybenzhydrylamine (**7**) was obtained from treatment of benzhydrylamine with di-*tert*-buty dicarbonate.[16] A slight amount of bis(*N*,*O*-Boc)-*p*-hydroxybenzhydrylamine was removed during crystallization. An overall yield of the three-step process to **7** was 37%. Throughout this synthesis, no attempt has been made to resolve the D or L isomer of **7** since the handle containing this center is eventually removed from the peptide product.

B. Attachment to the Aminomethyl Resin. The benzhydrylamine handle was then converted to a carboxylic acid derivative (Figure 3) which could be efficiently coupled by acylation to the aminomethyl resin. Esterification of handle **7** to glutaric anhydride gave *N*-Boc-*p*-glutaroylbenzhydrylamine (**8**) (method D). Under such conditions, all intermediates were purified before their attachments to the resin.[20] The coupling yields of handle **8** via DCC to give the resin **9** using this approach were

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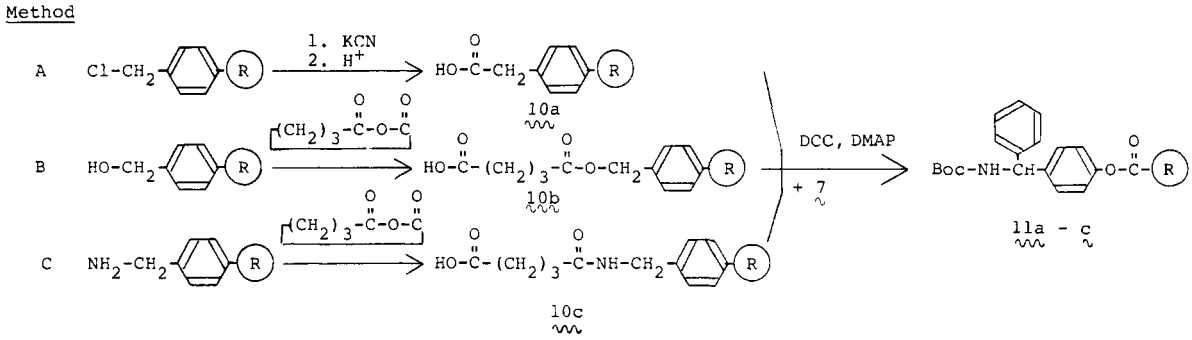

Figure 4. Preparation of *N*-Boc-*p*-(acyloxy)benzhydrylamine resin from *N*-Boc-*p*-(glutaroyloxy)benzhydrylamine (method d).

invariably high and averaged 99%. This method of preparation is chemically defined and is the preferred route to prepare the *p*-(acyloxy)benzhydrylamine resin.

C. Alternative Routes to the Preparation of *p*-(Acyloxy)benzhydrylamine Resin. *p*-(Acyloxy)-benzhydrylamine resin could also be achieved by esterification of *N*-Boc-*p*-hydroxybenzhydrylamine (**7**) through the phenolic moiety to a carboxymethyl resin **10a–c** (Figure 4). Carboxymethyl resin **10a–c** was prepared by three different routes (methods A–C) and from three different easily available resins: chloromethyl, hydroxymethyl, or aminomethyl resin. Conversion of the chloromethyl resin to carboxymethyl resin **10a** was effected by the "naked" cyanide displacement of chloride and by subsequent acid hydrolysis of the nitrile moiety to the carboxylic acid.[17] However, the overall yield of this two-step conversion was usually 70% based on the original chloride substitution (method A). Hydroxymethyl resin was obtained by potassium fluoride[18] catalyzed displacement of chloromethyl resin in acetic acid and subsequent removal of the acetyl group by hydrazinolysis.[19] Aminomethyl resin was prepared in a two-step process from the acid-catalyzed reaction of *N*-(hydroxymethyl)phthalimide.[20]

Carboxymethyl resins **10b** and **10c** were converted from the hydroxymethyl resin (method B) and the aminomethyl resin (method C) by acylation with glutaryl anhydride. Unlike carboxymethyl resins **10a** and **10c**, carboxymethyl resin **10b**, when attached to the benzhydrylamine handle **7**, contains three cleavable linkages: a benzhydrylamine, a phenyl, and a benzyl ester. Selective cleavage of the benzyl ester linkage could be envisioned (e.g., the S_N2 deprotection method of HF-DMS)[4,21] to give a peptide containing a carboxyl handle and would be particularly useful for reattachment to the aminomethyl resin for fragment synthesis. However, the benzyl ester linkage of this resin would also be more susceptible to TFA and thus would not be suitable for a long synthesis.

Attachment of the *N*-Boc-*p*-hydroxybenzhydrylamine to carboxymethyl resin **10a–c** was carried out in DCC with (*N*,*N*-dimethylamino)pyridine[22] as catalyst in a mixed solvent of CH_2Cl_2–DMF (1:1, v/v) to give the desired *N*-Boc-*p*-(acyloxy)benzhydrylamine resin **11a,b**. The yield ranged from 85 to 90%. Probably 10 to 15% of the carboxymethyl sites was converted as *N*-acylurea during the esterification, but this was presumed to be harmless. Two precautions were taken to ensure that unreactive carboxymethyl sites were removed completely in the resin. An excess amount of the phenolic component was used during the esterification to force the reaction to completion. Furthermore, at the completion of the reaction a 10-fold excess of phenol in DCC in the presence of DMAP was used to cover the remaining carboxylic acid sites.

Table I. Acidolytic Loss of Amino Acid from *N*-Boc-(aminoacyl)-*p*-(acyloxy)benzhydrylamine Resin (ABA Resin) in CF_3CO_2H Treatment[a]

amino acid resin	source	loss (mol %)[b] 24 h	36 h	48 h	rate,[c] 10^8k, s^{-1}	loss (mol %) per synth cycle (30 min[d])
Boc-Gly-ABA-R	**11a**	0.40	0.66	0.96	5.17	0.009
	9	0.44	0.70	0.81	4.83	0.009
Boc-Ala-ABA-R	**11a**	0.72	1.02	1.51	8.33	0.015
	9	0.69	0.89	1.02	6.74	0.012
Boc-Val-ABA-R	**11a**	0.52	0.75	1.18	6.46	0.012
	9	0.43	0.63	0.98	5.21	0.009
Boc-Phe-ABA-R	**11a**	0.22	0.37	0.52	2.94	0.005
	9	0.13	0.21	0.32	1.83	0.003

[a] CF_3CO_2H–CH_2Cl_2 (1:1, v/v). [b] Determined by amino acid analysis from the TFA filtrates. [c] Pseudo-first-order constant. [d] 30-min deprotection cycle to remove Boc group.

Stability of *p*-(Acyloxy)benzhydrylamine Resin. A. Effect of TFA and HF. Four Boc-(aminoacyl)-*p*-(acyloxy)benzhydrylamine resins derived either from carboxymethyl resin **11a** (method A) or from resin **9** (method D) even though both resins are apparently identical. Resin **9** is obtained from an unambiguous route with the intermediates purified before attachment to the resin support. Resin **11a** is prepared by a more general approach, but steps leading to the same final product are carried on the resin support and the intermediates are not purified. It is therefore important to demonstrate that by either approach the product will have the same stability and susceptibility toward CF_3CO_2H and HF. *N*-Boc-(aminoacyl)-*p*-(acyloxy)benzhydrylamine resins **12–15** were evaluated for their acid stabilities in TFA and for their susceptibility in HF. The *p*-(acyloxy)benzhydrylamine resin has two susceptible cleavage sites in TFA, the benzhydrylamine and phenyl ester linkages. Loss of amino acids or peptide chain could result from TFA-catalyzed solvolysis of either site. However, the phenyl ester linkage is stable to anhydrous HF, and only the benzhydrylamine linkage is susceptible to the anhydrous HF cleavage.

Treatment of these resins with 50% TFA in CH_2Cl_2 at 25 °C for 24–48 h produced only 1–2% loss of amino acids (Table I). Thus, the average loss per synthetic cycle of a 30-min TFA deprotection was 0.003–0.015%. There was little difference between the resin prepared from either method A or D. However, Phe-ABA resin was found to be 3–5 times more stable in TFA than the aliphatic amino acid counterparts such as Gly,Ala, or Val-ABA resin. These results are consistent with other findings that the linkage between the resin support and a C-terminal aromatic amino acid residue such as Phe is usually more stable to acidic cleavage than the corresponding aliphatic amino

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Table II. Comparison of Yields by the Conventional HF Cleavage and by the Gradative Deprotection Method on Boc-(aminoacyl)-*p*-(acyloxy)benzhydrylamine Resin

	cleavage yield,[b] %			
	HF[c]		gradative method[d]	
sample[a]	11a	9	11a	9
Boc-Gly-ABA-R (12)	85	84	98	99
Boc-Ala-ABA-R (13)	81	85	97	98
Boc-Val-ABA-R (14)	76	71	98	98
Boc-Phe-ABA-R (15)	69	66	98	97
Boc-Gly-Trp(For)-Met(O)-Asp(OBzl)-Phe-ABA-R		65		98

[a] ABA = *p*-(acyloxy)benzhydrylamine resin. [b] Yield obtained from 6 N HCl hydrolysis to the resulting resin. [c] HF–*p*-cresol (9:1, v/v) 0 °C, 1 h. [d] Low HF cleavage, then hydrazinolysis of the resulting resin (see the Experimental Section).

Table III. Basic and Nucleophilic Cleavage Methods for the Boc-(aminoacyl)-*p*-(acyloxy)benzhydrylamine Resin

	cleavage yield,[b] %			
sample[a]	Bu_4NCN[c]	NaOH[d]–H_2O_2	NH_2NH_2[e]	$HONH_2$[b]
Boc-Gly-ABA-R	73	75	99	92
Boc-Ala-ABA-R	71	73	98	90
Boc-Val-ABA-R	69	74	98	86
Boc-Phe-ABA-R	68	73	97	85

[a] Prepared by method D. [b] Yield based on amino acid analysis. (mol released from resin)/(mol on untreated resin) × 100. [c] Bu_4NCN–CH_3OH–DMF. [d] pH 10.6. [e] 5% hydrazine/DMF. [f] 10% hydroxyamine–DMF.

acid resin linkages.[12] Another susceptible site of the ABA resin to the acidic cleavage is the phenyl ester linkage to the conventional phenolic ester. The ABA resin was found to be 10 times more stable in 50% TFA than the conventional phenolic resin.[23] Again, the observed greater acidic stability may be attributed to the electron-withdrawing effect of the *p*-benzamido substituent on the ABA resin.

In parallel experiments, each resin sample was treated with HF–*p*-cresol (9:1, v/v) for 1 h at 0 °C.[24-26] The cleavage yield of the glycyl and alanyl resins was about 80%, but somewhat lower for the valinyl and phenylalanyl resins (Table II). Again, there was little difference in the *p*-(acyloxy)benzhydrylamine resin **11** or **9**. Thus, it can be concluded that the *p*-(acyloxy)benzhydrylamine resins, whether prepared from the carboxymethyl resin **10**, or the aminomethyl resin **9**, possessed the required stability toward 50% TFA and sufficient lability toward HF for the solid-phase synthesis.

B. Effect of Base and Nucleophile. A distinctive feature of *p*-(Acyloxy)benzhydrylamine resin is the presence of a phenyl ester linkage that confers its lability to the base and nucleophile. A more unusual feature of this linkage is that the ester is not a phenyl ester of the peptide component, but a retro phenyl ester in which the carboxyl group is part of the resin support. The versatility of the retro phenyl ester design allows *p*-hydroxybenzhydrylamine peptide **3** to be produced whether the reagent is hydrazine, hydroxylamine, cyanide, or peroxide. Cleavage yields of Boc-(aminoacyl)-*p*-hydroxy-benzhydrylamine resin obtained from method D by these reagents were between 68 and 99% (Table III). In general, the cleavage by hydrazinolysis proved to be most effective and required 1.0 h or less to give nearly quantitative cleavage of the handle from the resin. In all cases, Boc-(aminoacyl)-*p*-hydroxybenzhydrylamine was stable and could be reattached to the carboxymethyl resin.[5,17] Such flexibility allows the preparation of protected peptides.

Table IV. Chain Loss of Ala-*p*-(acyloxy)benzhydrylamine Resin during Neutralization and Coupling Step

	loss, mol %	
condition	24 h	per synth cycle
5% DIEA–CH_2Cl_2	3.2	0.02[a]
5% DIEA–DMF	12.7	0.09[a]
5% TEA–CH_2Cl_2	26.5	0.18[a]
HOBt–DMF[b]	42.4	1.79[c]
HOBt–DMF–Ac_2O[b]	2.2	0.09[c]
pyridine–DMF[b]	73.1	3.04[c]
pyridine–DMF–Ac_2O[b]	4.4	0.18[c]

[a] 10-min neutralization cycle loss determined by ninhydrin test. [b] 3 equiv of HOBt, pyridine, or Ac_2O to the resin. [c] 1-h coupling cycle, loss determined by amino acid analysis of the resin.

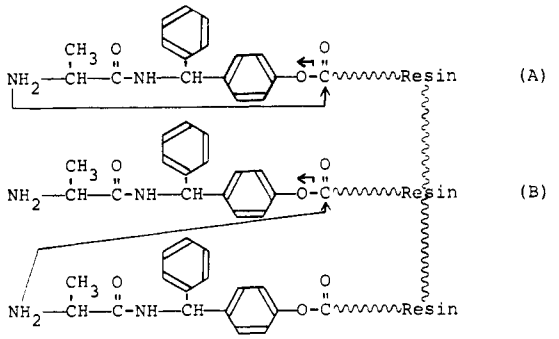

Figure 5. Intrachain (A) and interchain reaction (B) between the amino group and the retro phenyl ester.

A concern of the *p*-(acyloxy)benzhydrylamine resin is its general susceptibility to base as well as base-catalyzed inter- or intrachain reaction of the α-amino group to the retro phenyl ester during the neutralization or coupling step (Table IV). Intrachain reaction between the retro phenyl ester linkage and the α-amine will lead to loss of an α-amino group as terminated product on the peptide resin chain (Figure 5). However, interchain reaction will lead to a termination product and the release of the peptide into the solution with the net loss of two α-amino groups. The inter- and intrachain reactions will therefore account for a total loss of three α-amino groups. A model using a neutralized alanyl-*p*(acyloxy)benzhydrylamine resin (**9**) was treated in CH_2Cl_2 for 24 h to observe the loss of amino groups and to test these possibilities. The loss of the amino group, as monitored by the quantitative ninhydrin test[27] would provide the amount of total loss due to termination reaction. Determination of alanine by acid hydrolysis of the soluble filtrate would account for the interchain loss (Table IV). The total loss of amino groups with 5% DIEA in CH_2Cl_2 for 24 h was 3.2%, of which 1.35% was derived from the interchain or base-catalyzed cleavage reaction. The loss of 12.8% was more substantial in 5% DIEA–DMF solution, and the interchain or basic cleavage reaction accounted for 5.4%. When the less hindered tertiary amine triethylamine was used, the loss of 26.5% was even more substantial (Table IV) and should

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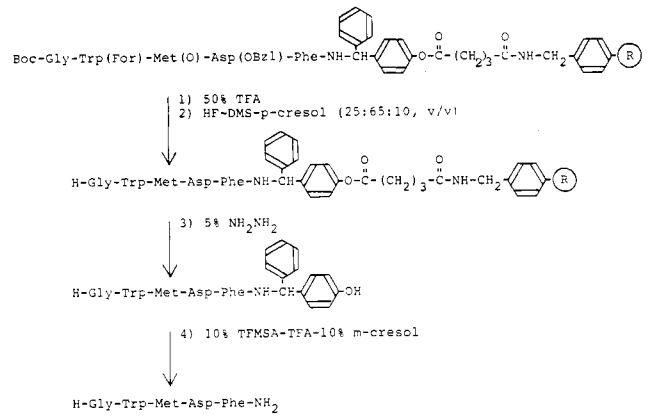

Figure 6. Gradative deprotection method of protected Boc-pentagastrin-*p*-(acyloxy)benzhydrylamine resin.

be avoided as the neutralizing agent. However, these losses would be small and inconsequential under the normal solid-phase condition in which the neutralization is conducted in CH_2Cl_2 for very brief periods (4–10 min/cycle).

Since nucleophiles or bases such as hydroxybenzotriazole (HOBt) or pyridine are sometimes present in the coupling step, the loss of amino groups in HOBt[28] or pyridine was also tested. As shown in Table IV, the loss of amino groups under these conditions was more severe. However, if acetic anhydride was added to simulate the actual coupling condition, the loss of the α-amino group was about 20-fold less than without the presence of the acylation agent. Since the coupling step is always in the presence of a reactive acylating agent, the loss of amino group per coupling step (1 h) will be small (<0.2%). These results showed that the retro phenyl ester linkage is susceptible to basic as well as nucleophilic reagents during the neutralization and the coupling steps. However, when proper precautions are taken, it has the stability required for stepwise synthesis by the solid-phase method.

Degradative Deprotection Approach and Application in Peptide Synthesis. To illustrate the utility of the *p*-(acyloxy)benzhydrylamine resin and the new approach to synthesize peptide amides, the C-terminal pentapeptide of gastrin, H-Gly-Trp-Met-Asp-Phe-NH_2, was prepared.[6c] The C-terminal pentagastrin is a potent active fragment of the gastrin hormones and is a well-characterized test peptide in our laboratory for new deprotection procedures.[4] It consists of an unusually rich concentration of difficult amino acids such as Trp, Met, and Asp and is a suitable model peptide for the efficacy of the new approach. The pentapeptide was synthesized by the stepwise approach using *N*-Boc-*p*-(acyloxy)benzhydrylamine resin prepared from method D (Figure 6) with Boc-Trp(For), Boc-Met(O), and Boc-Asp(OBzl) as starting materials. The protected pentagastrin resin was treated briefly with TFA to remove the N^{α}-Boc protecting group and then for 2 h at 0 °C with the "low HF" concentration procedure, which removed protecting groups by a mixture of HF–dimethyl sulfide–*p*-cresol–*p*-thiocresol (25:65:8:2, v/v). The mild "low HF" procedure removes all benzyl protecting groups by an S_N2 mechanism and concomitantly converts Trp(For) and Met(O) to Trp and Met. However, the *p*-(acyloxy)benzhydrylamine resin is stable under such a treatment, and the completely deprotected pentapeptide, H-Gly-Trp-Met-Asp-Phe, remained on the resin support.

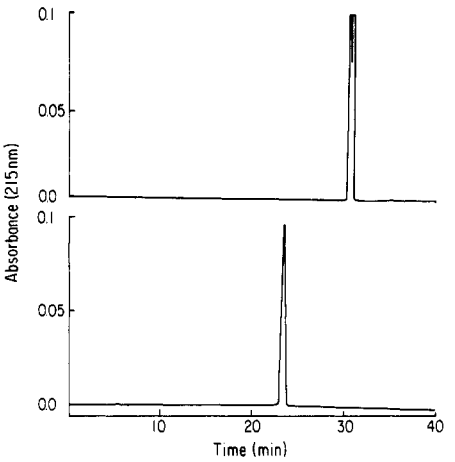

Figure 7. Analysis of crude and unpurified pentagastrin-*p*-hydroxybenzhydrylamine (A) and pentagastrin (B).

Table V. Removal of the *p*-Hydroxybenzhydryl Handle and Protecting Groups from Arg(Tos) and Cys(4-MeBzl) in Dilute Solution of a Strong Acid

	p-HOBzh-handle		rem, %/h	
condition[a]	% rem[b] after 1 h	time,[c] h, for 100% rem	Arg(Tos)	Cys(4-MeBzl)
CF_3CO_2H	<1		<0.1	<0.1
1% CF_3SO_3H	85	2	14.2	<0.1
2% CF_3SO_3H	100	1	24.2	<1
2.5% CF_3SO_3H	100	0.5	59	<1
3.0% CF_3SO_3H	100	0.5	65	<1
5.0% CF_3SO_3H	100	<0.25	77	1.2
7.5% CF_3SO_3H	100	<0.25	94	4.8
10% CF_3SO_3H	100	<0.1	99	9.7
4 N HBr		6	<1	<1

[a] All contain 10% *m*-cresol at 24 °C. [b] Determined by RP HPLC. [c] Determined by amino acid analysis.

Extraction of the peptide resin with solvent mixtures of mercaptoethanol–ether and DMF removed all the extraneous impurities and aromatic scavengers such as cresol and thiocresol from the resin. Brief treatment of the peptide resin with 5% hydrazine in DMF released from the resin support the pentapeptide with a racemic *p*-hydroxybenzhydrylamine handle. After neutralization of the cooled hydrazine solution with glacial HOAc and subsequent removal of the DMF in vacuo, the crude and unpurified product was analyzed by reversed-phase HPLC (Figure 7). Two symmetrical peaks, which accounted for 99% of the peptide product, were found. The symmetrical peaks were expected because the pentapeptide was attached to a racemic *p*-hydroxybenzhydrylamine handle to give diastereomers that were usually resolvable by HPLC. Treatment of the crude peptide with 1% TFMSA in TFA solution containing 10% *m*-cresol at 0 °C for 2 h as scavenger smoothly removed the *p*-hydroxybenzhydrylamine handle. Other conditions such as 4 N HBr–TFA 30 and various concentrations of TFMSA, to remove the *p*-hydroxybenzhydryl handle were also explored (Table V). However the dilute 1 or 2% TFMSA solution was found to be most satisfactory. Under such conditions aspartimide formation of the gastrin pentapeptide was not detectable.[4] The crude and unpurified product after extraction with chloroform to remove the aromatic compounds was analyzed by HPLC (Figure 7). A single symmetrical peak of the pentagastrin, accounting for 98% of the peptide content, was obtained.

In the gradative deprotection approach, the removal of the side-chain protecting groups is limited to those that

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are removable under the S_N2 conditions. Residues such as Arg(Tos) and Cys(MeBzl) are not removed under the recommended conditions. As shown in Table V, the tosyl group of Arg was removable with 5% TFMSA solution and Cys(MeBzl) in 10% TFMSA. To be fully compatible with the gradative deprotection approach, different protection group strategies for these two amino acid residues should be employed. The more acid-labile substituted tosyl protecting groups for arginine[31] could be a possible and useful solution. Similarly, the *S*-acetamido[32a] protecting group, which is removable by iodine,[32b] should also be considered for the sulfhydryl protecting group of cysteine. These two modifications would then avoid the more acidic condition during the final cleavage of the handle.

Discussion

The syntheses of peptide amides have been achieved using benzyl,[33-36] substituted benzyl, and phenyl esters[34] as well as benzhydrylamine linkages anchored on solid supports. The rate of ammonolysis of benzyl ester attached to the polystyrene resin is usually slow,[33] particularly when the hindered amino acid is at the C-terminus. Ammonolysis of benzyl ester attached to the more favorably solvated polyamide resin shows considerable improvement.[34] Better yields and reactivities are also seen with the more reactive phenyl ester resin. However, methods using ammonolysis often require special precautions to avoid the side product of peptide with free carboxylic acid. On the other hand, strongly acidic cleavage of the benzhydrylamine resin to give peptide amides is usually accompanied by several undesirable side reactions. Furthermore, the conventional preparation of benzhydrylamine directly on the polystyrene resin also produces uncertainties and unknown side reactions.[8] Thus, the multidetachable *p*-(acyloxy)benzhydrylamine resin prepared from a chemically unambiguous route and removalbe by dilute strong acid is designed to meet most of the favorable advantages desirable for the preparation of a peptide amide.

The *p*-(acyloxy)benzhydrylamine resin is designed with the concept of exploiting the chemical and electronic effects of one cleavage site to affect the lability of the other site. Similar concepts of manipulating electronic properties of substitution through the phenyl ring to affect its chemical reactivities in peptide synthesis has also been used.[37-39] This unusual design and properties of the multidetachable *p*-(acyloxy)benzhydrylamine resin and combination of the newly developed S_N2 deprotection method[4,21] by HF or (trifluoromethyl)sulfonic acid in dimethyl sulfide provide an alternative but selective deprotection approach in the synthesis of peptide amides. In this approach, strongly acidic anhydrous concentrated HF is completely avoided. Thus, the usual one-step, S_N1 cleavage process, which requires an acidity function between −9.5 and −11 to remove the peptide from the benzhydrylamine is substituted by three acidolytic steps with an acidity function between −1.5 and −6.5 in the gradative deprotection approach. The removal of N^{α}-Boc group by 50% TFA in CH_2Cl_2 has a calculated acidity function about −1.5 while the S_N2 removal of the benzylic protecting group is between −5.2 and −6.0. Finally, the solvolytic removal of the *p*-hydroxybenzhydrylamine handle in 1% TFMSA in TFA is calculated to have an acidity function of −6.0 to −7.0. These deprotection steps carried in greatly reduced acid strength will likely avoid many alkylation and acylation side reactions associated with the S_N1 process in excessively strong acidic conditions.

This principle has been applied successfully in the synthesis of many peptide amide hormones in high yield and high purity (unpublished). In the present example of synthesis, crude pentagastrin was obtained in an overall yield of greater than 90%. The cleavage yield from the resin support was 98%, which was considerably higher than the usual cleavage method of about 50% on the conventional benzhydrylamine. More importantly, the purity of the crude peptide was greater than 95%. Since many hormones are peptide amides, the gradative deprotection method will be a useful approach to obtain many of such compounds with high yields and high purities.

Experimental Section

Commercial protected amino acids were obtained from Peninsula Laboratories, San Carlos, CA: trifluoroacetic acid, Halocarbon Products; HF, Matheson; diisopropylethylamine and pyridine, Aldrich, both distilled over ninhydrin, Pierce; dicyclohexylcarbodiimide, Pierce; 1-hydroxybenzotriazole, Aldrich, recrystallized; dichloromethane, Eastman, distilled from Na_2CO_3; acetonitrile, HPLC grade, Jackson and Burdick.

Hydrolysis of free peptides was with 6 N HCl in evacuated, sealed tubes at 110 °C, 24 h. Peptide resins were hydrolyzed in 12 N HCl phenol–HOAc (2:1:1) at 110 °C, 24 h, and by 4 N methanesulfonic acid at 110 °C, 24 h. Samples were analyzed on a Beckman 121 amino acid analyzer. HF reactions were carried out in a Diaflon HF apparatus (Toho Co., Osaka, Japan).

Analytical high-pressure liquid chromatography of the peptides was on a reversed-phase μBondapak C-18 column (3.9 × 300 mm) in a Waters Associates instrument fitted with a Schoeffel variable-wavelength UV detector and an automatic Wisp injector. The chromatograms were recorded on a Hewlett-Packard 3380 A integrator (1-mV full scale). Elution conditions: linear gradient, 2% B to 98% B in 45 min at 215 nm, 0.1 AUFS; buffer A (0.1%), H_3PO_4 (95%)–CH_3CN(5%); buffer B (0.1%), H_3PO_4 (50%)–CH_3CN (50%); flow rate 2 mL/min.

Thin-layer chromatography (TLC) on silica gel plates (GF 250 μm) was developed with the following solvent system: CMA (chloroform–methanol–acetic acid, 85:10:5); CM (chloroform–methanol, 98:2); CA (chloroform–acetic acid, 95:5).

Preparation of D,L-*p*-Hydroxybenzhydrylamine. This compound was prepared in two steps:

Leukart Reductive Amine Reaction.[13] Into a 1-L round-bottom flask equipped with a still head carrying a thermometer and connected to a vertical condenser with a bent cold finger leading the condensate into a Dean–Stark receiver or directly back to the round-bottom flask were added 4-hydroxybenzophenone (8.5 g, 0.44 M Fluka), ammonium formate (110 g, 1.76 M), 98% formic acid (50 mL), and formamide (350 mL). The mixture, deaerated with nitrogen and immersed into an oil bath heated to 178–180 °C, was vigorously stirred, and yellowish condensate was collected into the Dean–Stark apparatus. After about 100 mL of condensate had been collected, the condensate with boiling point higher than 115 °C was directed back into the reaction flask and the reaction continued for 12 h. TLC indicated greater than 95% of the starting material had disappeared. The reaction mixture was cooled to about 120 °C and poured into 1 L of cold

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water. When left standing in the cold, the organic layer solidified as an off-white precipitate that was recrystallized in hot water to give a white crystalline solid. The yield was 87% (86 g). TLC (I_2 and UV_{254nm} detection; R_f): CMA, 0.55, starting material 0.73; CA, 0.07, starting material 0.32; CM, 0.50, starting material 0.72. Anal. Calcd: C, 73.99; H, 5.77; N, 6.16. Found: C, 74.11; H, 5.72; N, 5.99. This material was used without further purification.

Mild Acid Hydrolysis. *N*-Formyl-*p*-hydroxybenzhydrylamine (37 g, 0.163 M) was hydrolyzed in 300 mL of 6 N HCl–HCO_2H–H_2O (1:1:1) for 0.8 h at 75 °C. The reaction was cooled immediately to 40 °C by addition of 300 mL of H_2O, and the volume was concentrated to about 100 mL. The solution was then extracted 3 times with chloroform. The combined aqueous layer was reduced to dryness. The resulting white solid after extraction with hot EtOAc was recrystallized from EtOH–H_2O to obtain 25.1 g (68% yield) of D,L-*p*-hydroxybenzhydrylamine hydrochloride, mp 168–170 °C (lit.[9] mp 68 °C). TLC (ninhydrin and UV_{254nm}; R_f): CMA, 0.35; CM (9:1), 0.37. Anal. Calcd for ($C_{13}H_{14}NOCl$: HCl salt): C, 66.24; H, 5.99; N, 5.94. Found: C, 66.34; H, 5.97, N, 5.90.

***N*-Boc-*p*-hydroxybenzhydrylamine.** Di-*tert*-butyl dicarbonate (Fluka, puriss, 18.53 g, 19.5 mL) was added dropwise for 1 h into a stirred solution of D,L-*p*-hydroxybenzhydrylamine hydrochloride (20 g, 84.92 mM) in 250 mL of 2-butanol and water (2:1) and sodium hydroxide (3.39 g, 84.92 mmol) at 0 °C. The reaction was maintained at about pH 8 by small addition of 1 N NaOH. After 3 h, the solution was acidified by potassium hydrogen sulfate to pH 4 and extracted three times with 150 mL of ethyl acetate. The combined ethyl acetate layer after being dried over sodium sulfate was decolorized in charcoal to give a yellowish solid after the removal of solvent. Crystallization in pentane in a crystallizing dish yielded 18.34 g of colorless solid (72%), mp 138–140 °C (lit.[9] mp 140–142 °C). TLC (UV_{254nm} and ninhydrin detection; R_f): CMA, 0.54 ^1H NMR (acetone-d_6) 1.43 (s, 9 H), 5.40 (m, 1 H), 5.80 (m, 1 H), 7.24 (s, 5 H), 7.7 (s, 1 H). Anal. Calcd for $C_{18}H_{21}NO_3$: C, 67.02; H, 6.75; N, 3.91. Found: C, 67.09; H, 6.78; N, 3.87.

Preparation of Carboxymethyl Resin. Method A. A slurry of finely ground anhydrous potassium cyanide (6.50 g, 100 mmol) and dicyclohexyl 18-crown-6 ether (5.8 g, 16.1 mmol; Fluka, purum grate) was added to a suspension of mechanically stirred (chloromethyl)polystyrene resin (50 g, 0.32 mmol/g substitution; Lab System) in *N*-methylpyrrolidone (250 mL) at 50 °C. Samples were retrieved at intervals to determine the amount of chloride left by the modified ninhydrin monitoring method for chloromethyl resin (see below). After 48 h the ninhydrin reaction was nearly negative. The reaction was stopped, and the resins were washed three times each with 250 mL of dimethylformamide–H_2O (1:1, v/v), dimethylformamide, glacial acetic acid–dioxane (1:1, v/v), and methanol. After drying, elemental analysis of nitrogen was 0.35% N, (0.25 mmol/g of substitution) and 0.13% Cl (0.036 mmol/g). IR (KBr) showed a weak nitrile band at 4500 cm^{-1}. The cyanomethyl resin was then hydrolyzed in 400 mL of concentrated HCl–phenol–acetic acid (2:1:1, v/v/v) for 24 h, after washing the resin three times each with 250 mL of H_2O, H_2O–tetrahydrofuran (1:1, v/v), acetic acid–tetrahydrofuran (1:1, v/v), tetrahydrofuran, and methanol. A slightly colored resin was obtained with no nitrogen found by elemental analysis. Chlorine analysis was 0.07 mmol/g of chloride but gave a negative result by our new ninhydrin method for chloromethyl resin. Thus, there remain some chloride sites that are not easily displaced and probably not of the chloromethyl origin. IR (KBr, cm^{-1}): 3100, 3060, 2950 (br, OH), 1700 (C=O), absence of the nitrile peak at 4500 cm^{-1}. Resins were then sized to remove fine as well as large aggregates in two solvents (methanol and methylene chloride), and 42 g of resin was obtained (84% yield).

Method B. Glutaryl anhydride (75 mg, 0.64 mmol) and (*N*,*N*-dimethylamino)pyridine (40 mg, 0.33 mmol) were added successively to hydroxymethyl resin (2 g, 0.30 mmol/g) suspended in 20 mL of CH_2Cl_2–DMF (1:1, v/v). After agitated by a mechanical shaker for 18 h at 24 °C, the resin was filtered and washed successively (three times each) with DMF, HCl in dioxane–H_2O (1:1, v/v), and CH_3CN. The dried resin in IR (KBr, cm^{-1}) gave a broad peak at 2950 and 1700.

Method C. Glutaryl anhydride (0.75 g, 6.4 mmol) was added to aminomethyl resin (5 g, 2.2 mmol) suspended in 100 mL of CH_2Cl_2–DMF (1:1, v/v). After agitation by a mechanical shaker for 4 h at 24 °C, the resin was filtered and washed successively (three times each) with DMF and CH_3CN. The dried resin was shown by the quantitative ninhydrin method to contain less than 0.2% of amino sites. IR (KBr cm^{-1}) showed a broad peak at 2950 and 1700.

Preparation of *N*-Boc-D,L-*p*-(glutaroyloxy)benzhydrylamine. Method D. To a dissolved CH_2Cl_2 solution (80 mL) of *N*-Boc-D,L-*p*-hydroxybenzhydrylamine (9.22 g, 30.8 mmol) and glutaryl anhydride (3.52 g, 30.8 mmol) was added (*N*,*N*-dimethylamino)pyridine (0.56 g, 4.62 mmol) at 0 °C. After 2 h a small portion of glutaryl anhydride (0.352 g, 3.08 mmol) was added to allow the reaction to completion. After 5 h the reaction was washed vigorously with 3 × 60 mL of 0.5 M $KHSO_4$. The CH_2Cl_2 layer was then dried over Na_2SO_4 and reduced in vacuo to syrupy oil that was redissolved in 50 mL of EtOAc and 150 mL of hexane and crystallized to a white solid in a crystallizing dish. Three crops were obtained to give 7.8 g (61.3% yield): TLC in CMA (85:10:5) R_f 0.58; mp 115–117 °C. Anal. Calcd for $C_{23}H_{27}NO_6$: C, 66.81; H, 6.58; N, 3.39. Found: C, 66.75; H, 6.52; N, 3.31.

Attachment of *N*-Boc-D,L-*p*-hydroxybenzhydrylamine to the Carboxymethyl Resin. *N*-Boc-D,L-*p*-hydroxylbenzhydrylamine (0.75 g, 2.5 mmol), dicyclohexylcarbodiimide (0.52 g, 2.5 mL), and (*N*,*N*-dimethylamino)pyridine (0.20 g, 1.64 mmol) were added successively (2 min apart with shaking) to the carboxymethyl resin (method A) (5 g, 1.25 mmol) in 50 mL of CH_2Cl_2–DMF (1:1, v/v). After 12 h, the reaction was terminated by the addition of 0.52 g of dicyclohexylcarbodiimide and 0.5 g of phenol for 1 h. The resins were washed three times each with 50 mL of dimethylformamide, acetic acid–methylene chloride (1:1, v/v), methylene chloride, and acetonitrile. Both elemental nitrogen analysis and quantitative ninhydrin analysis (after removal of the Boc group and neutralization of the TFA salt by DIEA) showed the substitution was 0.25 mmol/g.

Attachment of *N*-Boc-D,L-*p*-(glutaroyloxy)benzhydrylamine to Aminomethyl Resin. *N*-Boc-D,L-*p*-glutaroylbenzhydrylamine (1.24 g, 3 mmol) and dicyclohexylcarbodiimide (0.62 g, 3 mmol) were added successively to the aminomethyl resin (5 g, 2.0 mmol) in 50 mL of CH_2Cl_2. After 2 h, the resin was washed (three times each) with DMF, CH_2Cl_2, HOAC–CH_2Cl_2 (1:1, v/v), CH_2Cl_2, and CH_3CN. Quantitative ninhydrin test showed that 99.5% of the amines was coupled.

Qualitative Detection of Chloromethyl Sites of Resin. A convenient qualitative detection based on the ninhydrin test was developed to monitor the displacement of chloride from the chloromethyl resin by potassium cyanide during the synthesis of the cyanomethyl resin, an intermediate for the synthesis of the carboxymethyl resin. Resins containing chloromethyl sites (1–5 mg) were placed in the bottom of a test tube (5 × 100 mm). Three drops of pyridine were added, and the mixture was heated in a heating block for 15 min at 110 °C. Two drops of reagents required for the quantitative ninhydrin method and in addition 0.2 mg of leucine were added to the reaction mixture, and the whole reaction was heated at 110 °C for another 10 min. The reaction was cooled in ice water; the intensely blue mixture was taken up in 2 mL of 60% EtOH, filtered, and washed three times with 2 mL of 60% EtOH. Positive chloride test was identified by an intense blue color on the resin. The blue color could be eluted off by the tetramethylammonium chloride in CH_2Cl_2 and diluted with 60% EtOH and read at A_{570nm}. The chloride value would correspond to the calculated value of the ninhydrin color eluted off from the resin bead.

Stability of Boc-(aminoacyl)-D,L-*p*-(acyloxy)-benzhydrylamine Resin in Trifluoroacetic Acid–CH_2Cl_2 (1:1, v/v). Samples of Boc-(aminoacyl)-D,L-*p*-(acyloxy)benzhydrylamine resin (100 mg each, 10**a**–**d**) prepared from methods A and D were placed in a reaction vessel with 10 mL of trifluoroacetic acid–CH_2Cl_2 (1:1, v/v) for the required time, and the resin was then washed three times with TFA–CH_2Cl_2 (1:1, v/v). The combined filtrates were evaporated, hydrolyzed in 6 N HCl for 24 h, and quantitated on a Beckman amino acid analyzer. The resulting resins were also dried, and the amino acid content was analyzed by the quantitative ninhydrin method. The amount of cleavage of amino acids due to the TFA–CH_2Cl_2 treatments was comparable as determined by both methods. The results are summarized in Table I.

Treatment of Boc-alanyl-*p*-(acyloxy)benzhydrylamine Resin with Base and Nucleophile. A. Stability Test. Samples of Boc-Ala-*p*-(acyloxy)benzhydrylamine resin (100 mg each, 0.4 mmol/g) prepared from method D were treated in separate experiments with 1-hydroxybenzotriazole, diisopropylethylamine, triethylamine, or pyridine in either CH_2Cl_2 or DMF. After 24 h, resins were washed with DMF, CH_2Cl_2, and CH_3CN, and Ala left on the resin was determined by the quantitative ninhydrin test after the removal of the (*tert*-butyloxy)carbonyl and neutralization by diisopropylethylamine. Filtrates were evaporated, hydrolyzed in HCl–propionic acid (1:1, v/v) at 130 °C for 4 h, and quantitated by the amino acid analyzer. The results are summarized in Table IV.

B. Cleavage Reactions. Alanyl resin (100 mg each, 0.4 mmol/g) were treated with 3 equiv each of 95% NH_2NH_2, $HONH_2$·HCl with Et_3N, H_2O_2–Na_2CO_3 (pH 11.5)–dioxane–H_2O (1:1), and tetrabutylammonium cyanide in 50% MeOH–DMF for 1 h at 24 °C. Analysis of the cleavage yield was similar for A. The results are summarized in Table III.

Similarly, resins **12–15** (100 mg each) were treated with 5 mL of HF–*p*-cresol (9:1, v/v) at 0 °C for 1 h. Separately, each resin was also treated with the gradative deprotection method of 5 mL of HF–dimethyl sulfide–*p*-cresol (25:65:10, v/v) for 2 h and washed with CF_3CO_2H–CH_2Cl_2 (1:1, v/v) 3× (2 min), CH_2Cl_2 3× (1 min), DMF 3× (1 min), 5% NH_2NH_2–DMF 1× (30 min), and DMF 3× (1 min). The resins resulted from both treatments were then hydrolyzed in 12 N HCl–phenol–HOAC (2:1:1, v/v) for 24 h. The cleavage yield from the resin was calculated by 100% – % remaining on the resin. The results are summarized in Table II.

Synthesis of Test Peptides. General Procedure. The syntheses of test peptides were carried out by stepwise solid-phase methods on *N*-Boc-*p*-(acyloxy)benzhydrylamine–copoly (styrene–1% divinylbenzene). Protected amino acids used were Asp(OBzl) Met(O), and Trp(For). Each synthetic cycle consisted of (i) a 20-min deprotection with 50% CF_3CO_2H–CH_2Cl_2, (ii) neutralization with 5% DIEA/CH_2Cl_2, and (iii) double coupling with preformed symmetrical anhydrides (3 equiv) for 1 h each in CH_2Cl_2 and recoupling in dimethylformamide. Boc-Gly was coupled with DCC alone. All couplings were monitored by the quantitative ninhydrin test.[27]

Pentagastrin Amide. The pentapeptide Boc-Gly-Trp-(For)-Met(O)-Asp(OBzl)-Phe-NH-CHC_6H_5–C_6H_4–$OCOCH_2$ resin was synthesized from N-Boc-*p*-(acyloxy)benzhydrylamine resin (method D; 2 g, 0.80 mmol).

Gradative Deprotection. The pentapeptide resin (0.50 g) was treated with the following protocol: (i) 50% CF_3CO_2H–CH_2Cl_2, 2× (5 min); (ii) CH_2Cl_2, 3× (1 min); (iii) 5% DIEA, 2× (1 min); (iv) CH_2Cl_2, 3× (1 min) (dried in vacuo); (v) HF–DMS–*p*-thiocresol–*p*-cresol (25:65:7.5:2.5, v/v, 10 mL), 1× (120 min), 0°C; (vi) ether–mercaptoethanol (95:5, v/v), 2× (2 min); (vii) CF_3CO_2H–anisole (9:1, v/v), 2× (1 min); (viii) 25% CF_3CO_2H–CH_2Cl_2, 2× (1 min); (ix) CH_2Cl_2, 3× (1 min); (x) 5% DIEA, 2× (1 min); (xi) DMF, 3× (1 min); (xii) 5% NH_2NH_2–DMF (4 mL), 1× (30 min); (xiii) DMF, 1× (1 min) (4 mL). The filtrates of steps xii and xiii, were collected, cooled, and neutralized dropwise with an equivalent amount of glacial acetic acid. In most cases, the peptides precipitated out after dilution with H_2O (20-fold). In such cases, the precipitate was collected, redissolved in 1–10% HOAc, and lyophilized to dryness. Otherwise, the DMF filtrate was reduced to dryness under vacuum to a white powder. The hydrazine salt and peptide were redissolved in the mobile phase containing 0.05% CF_3CO_2H and 5% CH_3OH, and the hydrazine acetate was removed through reversed-phase HPLC. Peptide products were then eluted by the mobile phase containing 80% CH_3OH.

The dried pentapeptide amide was then treated with (xiv) 1% CF_3SO_3H–CF_3CO_2H with 8% *m*-cresol and 2% DMS (2 mL) for 2 h at ambient temperature (or 2% CF_3SO_3H–CF_3CO_2H with 8% *m*-cresol and 2% DMS for 1 h) and (xv) diluted with 10-fold ether, cooled in dry ice bath. If there was no precipitation, pyridine was added (~1%). The precipitate was collected, centrifuged, washed with ether, and dried in vaccuo. It was redissolved and lyophilized in 1–10% HOAc. (Note: step (v) *p*-thiocresol is not necessary if Trp(For) is not present. In such cases, the mixture will be HF–DMS–*p*-cresol (25:65:10, v/v.)

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Formal Steric Enthalpy. Alcohols, Ethers, and Olefins[1,2]

DeLos F. DeTar,* Seyhun Binzet, and Prashanth Darba

Department of Chemistry, The Florida State University, Tallahassee, Florida 32306

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Formal steric enthalpy (FSE) is a general quantitative measure of the steric properties of a single conformer of a molecule. In this study we develop FSE values for alcohols and ethers and for olefins, providing for the first time a formally defined (though initially unknown) quantitative measure of the steric properties of these key molecules. We have calculated formal steric enthalpies for representative examples. The concept and the procedures may be extended to other classes of compounds.

There has been an accelerating interest in the use of computer techniques based on molecular mechanics for evaluating steric influences on reactions. A serious limitation in such applications has been the lack of a theoretically valid quantitative measure of steric effects. Raw steric energies as derived from molecular mechanics calculations are of limited utility since SE's are force field dependent and have no theoretical significance. Pairwise comparisons of SE values are valid only between isostructural molecules such as conformers and are useless for comparisons among members of general families of molecules.

We have developed a new measure of steric properties that overcomes these limitations for many classes of molecules and have called it formal steric enthalpy (FSE).[3]

(1) Definitions: SE, steric energy as calculated by molecular mechanics; FSE, formal steric enthalpy as defined in the present study; DETSB, the DeTar–Binzet force field developed in the present study; WHI77, the White 1977 force field.[2]

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