Quantitative Removal of a Pentadecapeptide ACTH Fragment Analogue from a Merrifield Resin Using Ammonium Formate Catalytic Transfer Hydrogenation: Synthesis of [Asp^{25},Ala^{26},Gly^{27},Gln^{30}]-ACTH-(25–39)-OH

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Ammonium formate catalytic transfer hydrogenation (CTH) has been used for the simultaneous deprotection and release of a pentadecapeptide analogue of ACTH ([Asp^{25},Ala^{26},Gly^{27},Gln^{30}]-ACTH-(25–39)-OH) from a Merrifield polystyrene resin. The yields were virtually quantitative, and the ultimate product (following N-terminal *tert*-butoxycarbonyl (Boc) group removal and gel filtration) compares favorably in both purity and yield with a purified sample obtained by hydrogen fluoride treatment. The peptide resin was prepared on a 0.77 mequiv/g scale by solid-phase methods and extended through 15 successive cycles of deprotection–coupling steps. A modified SPPS protocol provided an excellent yield of peptide resin with little evidence of heterogeneity. Following acidolytic (HF) and hydrogenolytic cleavages, the Boc-protected and deprotected products were compared by high-performance liquid chromatography, and the structures were confirmed by amino acid analysis and by fast-atom-bombardment mass spectroscopy. When hydrogenolysis was carried out without added acetic acid, a second product was isolated that contained one remaining benzyl protecting group, presumably the partially deprotected benzylserine moiety. With use of a "one-pot" procedure with ammonium formate CTH, and added palladium hydroxide on carbon and acetic acid, followed by trifluoroacetic acid treatment (to remove the Boc group), a fully deprotected peptide was obtained, in 94% yield, conforming in all respects to the authentic sample. These results suggest that the removal of large peptides from polystyrene supports using neutral or mildly acidic conditions is compatible with the most standard version of the solid-phase method.

A nearly universal problem in heterogeneous support systems for solid-phase peptide synthesis[1a] (Merrifield SPPS[1b]) involves the final step of the synthesis, in which the desired product is detached from the resin. While acid-based treatments are most commonly employed (HF,[2] HBr–TFA,[3] CF_3SO_3H–TFA,[4] and $B(O_2CCF_3)_3$,[5]), these procedures are often deleterious when acid-sensitive functionalities are present. The native lysozyme, when exposed to conditions for anhydrous HF cleavage, lost 87% of its biological activity.[6] The reasons for this are as yet unclear but are at least partly related to N $\rightarrow$ O migrations with serine moieties and other carbocation-mediated side-reaction products.[7–9]

Numerous alternatives to the most commonly employed Merrifield techniques have been proposed, including changes in the type of support used (from polystyrene-based to polyamides[10] or polyhalocarbons[11]), liquid-phase methods,[12] liquid–solid-phase systems,[13] multidetachable links,[14] photolabile links,[15] and various types of transesterification reactions for removal of the peptides under more gentle conditions.[16] To date, however, none of these alternatives have superseded the essential Merrifield method.

An attractive alternative for peptide–polymer cleavage, yet one fully compatible with the polystyrene support system, is represented by the hydrogenation technique. Among its advantages are that it would (1) protect chiral amino acids from racemization, (2) have the added advantage of leaving acid-labile protecting groups intact, and (3) be compatile with subsequent fragment condensation strategies.

The earliest report of hydrogenolysis of peptide resin benzyl esters using conventional hydrogenation techniques was by Schlatter et al.[17a] Thus, Boc-protected [Val^5]-enkephalin was obtained in 71% yield by treating the peptide resin with hydrogen gas at 40 °C and 60 psi for 24 h in the presence of palladium black generated in situ as catalyst.[17b] Khan and Sivanandaiah successfully cleaved Boc-bradykinin from its resin in 60% yield by using catalytic transfer hydrogenation (CTH) with cyclohexene as the hydrogen donor.[18] This reaction, while carried out at

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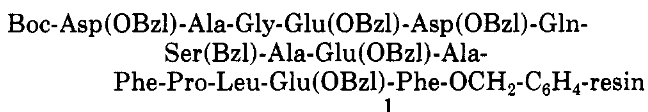
Table I. Methodology for the Solid-Phase Peptide Synthesis of [Asp[25],Ala[26],Gly[27],Gln[30]]-ACTH-(25-39)-OH

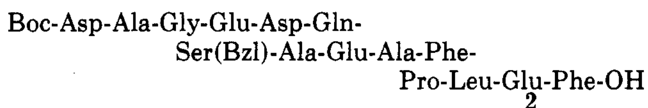
step no.	solvent	volume, mL	repetitions and time, min
1	50% (v/v) toluene–TFA	300	1 × 1, 1 × 30
2	toluene	200	2 × 1
3	20% (v/v) methanol in toluene	200	3 × 1
4	toluene	200	2 × 1
5	5% (v/v) diisopropylamine in toluene	200	1 × 1
6	methylene chloride	200	2 × 1
7	5% (v/v) diisopropylamine in methylene chloride	200	1 × 1, 1 × 3
8	methylene chloride	200	3 × 1
9	solvent to be used in coupling reaction	200	3 × 1
10	coupling with active ester solution	200	overnight
11	solvent used for coupling	200	2 × 1
12	methanol	200	3 × 1
13	toluene	200	4 × 1

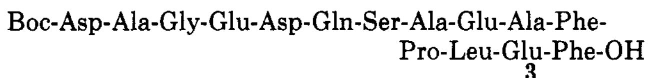
70 °C, now required a considerably shorter reaction time of 4 h. Although it has been suggested that the conventional hydrogenation technique is apparently not suitable for longer peptides,[19] recent advances in CTH methods predicted their potential use in such applications.[20]

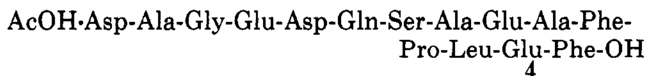
We recently reported an extremely rapid CTH reaction, carried out under neutral, ambient conditions, that utilizes ammonium formate as the in situ hydrogen source.[21] This reaction was then applied to peptide–resin cleavages and was used for the quantitative cleavage of leucine-enkephalin from a 1% divinylbenzene cross-linked polystyrene resin in under 2 h.[22] This result, however, while promising, did not address the concern regarding the lack of methods available for cleaving large, more complex peptides. We now report our results with a pentadecapeptide fragment, an ACTH-(25-39) analogue (1), (Chart I), first under identical conditions as used in our initial studies, and then with improved conditions suitable for benzyl ether functions.

Chart I









Results and Discussion

The ACTH fragment was synthesized on a polystyrene support cross-linked with 1% divinylbenzene, by using the rather high initial substitution value of 0.77 mequiv of the C-terminal phenylalanine/g of resin. The solid-phase synthesis was carried out by using the protocol described in Table I. Each step was monitored after both deprotection and coupling by the ninhydrin test of Kaiser et al.[23] Following each acid treatment, an aliquot of peptide–resin was removed and the neutralized peptide acetylated with acetic anhydride. This was then resubjected to acid treatment and monitored by the ninhydrin test; a positive reaction at this point indicates residual Boc groups, requiring an additional acid-treatment cycle. A negative test permitted the SPPS to continue with the subsequent condensation, with pre-formed HOBT "active ester" in 2-fold excess, in a mixture of toluene and DMF as the solvent medium. If recoupling was required, then the symmetrical anhydride of the Boc-amino acid, 1.5 equiv. was employed, with methylene chloride as solvent.

Table II. Amino Acid Analysis Results of ACTH-(25-39) before and after Cleavage from Resin

	expected	found: purified product	found: peptide resin
Asp	(2)	1.93	1.94
Ser	(1)	0.88	0.58
Glu	(4)	4.11	4.03
Pro	(1)	1.01	1.01
Gly	(1)	0.98	0.97
Ala	(3)	3.02	2.98
Leu	(1)	1.02	1.07
Phe	(2)	1.91	2.01

Amino acid analyses of the completed peptide resin (and of the final purified product) are shown in Table II. When subjected to HF treatment for cleavage and deprotection using standard conditions of 45 min at 0 °C followed by gel filtration, the resulting product was isolated in 90% yield.

For cleavage of the peptide by hydrogenolysis, the initial conditions employed were identical with those used for the removal of leucine-enkephalin from the Merrifield peptide resin.[22] The palladium black was deposited onto the polystyrene resin by diffusing the palladium acetate dissolved in DMF into the swollen resin beads for 2 h, after which a solution of ammonium formate in water was added, reducing the palladium(II) acetate and resulting in an even deposition of catalyst throughout the beads. Since it was known from earlier studies that the serine benzyl function is deprotected more rapidly with acid than under neutral conditions,[21] but in any case deprotection being much slower than it is with benzyl esters or phenolic benzyl ethers, the behavior of this moiety to the heterogeneous conditions of solid-phase hydrogenolysis was unknown.

In fact, after workup of the reaction and isolation of the peptide fraction, TLC analysis showed the product to consist of two major components with virtually no other observable byproducts. The minor product at higher R_f value was suspected of being a partially deprotected peptide, most likely having but one benzyl group remaining at serine. This was further supported by mass spectral analysis (fast atom bombardment) of the two fractions, a mixture of **2** and **3** and **3** (obtained from a reduction of **2**), which showed parent ions (±2 amu) as positive ions at m/e 1820 and 1728 and negative ions at m/e 1818 and 1726, for **2** and **3**, respectively. The difference in mass between the two molecular ions corresponds to the presence of one

(19) Reference 1, p 80.
(20) A recent successful application of catalytic transfer hydrogenation for the cleavage of an octacosapeptide using repeated hydrogenolysis with cyclohexadiene and a large excess of catalyst was reported by R. Colombo, *J. Chem. Soc., Chem. Commun.*, 1012 (1981).
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Table III. HPLC Characterization of the Solid-Phase Ammonium Formate CTH Products[a]

compound	retention time, t_R, min	k' values[d]
AcOH·Asp-Ala-Gly-Glu-Asp-Glu-Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe-OH (4)	10.85[b]	6.05
	8.78[c]	4.85
authentic sample of 4 (obtained by HF cleavage of 1)	10.66[b]	6.06
	8.78[c]	4.85
Boc-Asp-Ala-Gly-Glu-Asp-Gln-Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe-OH (3)	16.79[b]	9.97[e]
	14.61[c]	8.74
Boc-Asp-Ala-Gly-Glu-Asp-Gln-Ser(Bzl)-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe-OH (2)	19.28[c]	11.85

[a] Conditions of chromatography: a linear gradient of acetonitrile (20–40% over 20 min) was run against 0.2 M triethylammonium phosphate buffer (pH 2.5) on a reversed-phase C-8 Zorbax column (4.6 mm × 250 mm) maintained in a thermostated oven at 50 °C. [b] Flow rate = 1.5 mL/min. [c] Flow rate = 2 mL/min. [d] Capacity factor k' defined according to equation $k' = t_R(\text{cmpd}) - t_R(\text{solvent})/t_R(\text{solvent})$, t_R(solvent) being the retention time for elution of an unretained solute. [e] A small amount of compound 4 (<5%, t_R = 10.88 min, k' = 6.11) was detected in the HPLC profile.

benzyl function; compound 3 showed a positive ion m/e 1728 and negative ion at m/e 1726 (molecular formula for compound 3, $C_{76}H_{108}N_{16}O_{30}$; formula weight, 1725.82).

With the assumption that the faster moving component on TLC represented the N^{α}-Boc, *O*-benzylserine-protected peptide, the mixture of products was subjected to solution CTH, using 10% palladium on carbon as catalyst in 50% aqueous acetic acid. The two products gradually coalesced to one, that having the lower R_f. It thus appears that all hydrogenolyzable protecting groups except for the serine benzyl ether were removed during cleavage of peptide from the resin. The product that was subjected to the CTH treatment also contained an N-terminal *tert*-butoxycarbonyl group (Boc-Asp). This group was essentially unaffected during the various hydrogenation steps but may have suffered minimal cleavage during subsequent acidolytic purifications and treatments. In any case a ^{1}H NMR spectrum of the final Boc-pentadecapeptide showed good agreement with respect to the ratio of diastereotopic methyl hydrogens of leucine to aromatic hydrogens of phenylalanine, giving a value of 6 to 9.5 (theory, 6:10).

The yield of the gel-filtered product isolated from ammonium formate CTH in the first trial was about 79% (not corrected for some loss due to solubility of product in water). Quantitative amino acid analysis confirmed that this consisted of >90% of peptide-related substances. When the ammonium formate CTH cleavage product was subsequently treated with trifluoroacetic acid in methylene chloride, a new peptide was obtained that corresponded exactly (Table III) to the fully deprotected material from HF cleavage.

The overall yields of purified peptides were approximately 90% and 78% from HF and ammonium formate CTH cleavages, respectively. It was anticipated that the latter yield would be improved if the number of manipulations, including separate steps for cleaving *O*-benzylserine and N-terminal Boc groups, were done in succession without isolation of the intermediates. The non-pyrophoric "Pearlman's catalyst" (20% palladium hydroxide on carbon) was used in place of 10% palladium on carbon. It has been reported that palladium hydroxide on carbon is a superior catalyst in the hydrogenolysis of the benzyl ethers of primary and secondary alcohols.[24,25] Model experiments were performed with Boc-Thr(Bzl)-OH as the substrate in ammonium formate CTH, employing palladium hydroxide on carbon as the catalyst; prolonged reaction times up to 24 h gave only approximately 50% of the hydrogenolyzed product under neutral conditions. However, when acetic acid was introduced into the reaction mixture, the reaction was almost quantitative in under 1 h. In contrast, with 10% palladium on carbon in the presence of acetic acid, the reaction required approximately 2 h.

Accordingly, the cleavage of the protected ACTH-(25–39) peptide resin was repeated by using the following conditions: after treatment of the peptide resin, (1.5 g) with palladium acetate and ammonium formate in dimethylformamide for 2 h, an equivalent volume of acetic acid and palladium hydroxide on carbon (0.5 g) was introduced and the reaction allowed to proceed for an additional 2 h. After standard workup (cf. Experimental Section) and removal of the Boc group with 50% TFA in CH_2Cl_2 (v/v) followed by chromatography on Sephadex G-25, the fully deblocked ACTH-(25–39)-OH analogue was isolated in 94% yield.

A further consideration in designing a one-pot deprotection/cleavage experiment involved the activity of the resin-deposited palladium as a deprotection catalyst (in contrast to the subsequently added palladium hydroxide). In order to evaluate its activity, palladium was deposited in situ on polystyrene cross-linked with 1% divinylbenzene (Bio-beads SX-1, Bio-Rad Products) and used as the catalyst in a solution-phase ammonium formate CTH reaction, employing *N*-(benzyloxycarbonyl)phenylalanine *tert*-butyl ester as the substrate. Reaction conditions corresponding to solid-phase ammonium formate CTH were simulated. Periodic TLC monitoring of the reaction mixture indicated a reaction time of nearly 60 min. In contrast, when 10% palladium on carbon or 20% palladium hydroxide on carbon was used as catalyst in the same model experiment, the reaction was complete in under 8 min. Thus, it appears that the addition of 20% palladium hydroxide on carbon to the solid-phase CTH reaction mixture, though not essential, may be preferable, particularly when the molecule contains refractory 1° and 2° alcohol benzyl ethers.

It has therefore been established that the ammonium formate CTH procedure is compatible for the deprotection and cleavage of even reasonably lengthy peptide sequences in short reaction times under exceptionally mild conditions in spite of the dual heterogeneity of catalyst and polymer support. The flexibility and selectivity offered by this technique should be compatible with the synthesis by fragment condensation of large peptides and, possibly, small protein sequences, using suitably protected fragments.

Experimental Section

Reagent-grade ammonium formate was used for the hydrogenation reactions. Palladium(II) acetate was supplied by Aldrich Chemical Co. TLC was performed on silica gel (Merck F 254 plates) in the following systems (v/v): (A) ethanol/water, 7:3;

(24) P. N. Rylander, "Catalytic Hydrogenation in Organic Synthesis", Academic Press, New York, 1979, p 280, and references cited therein.
(25) S. Hanessian, T. J. Liak, and B. Vanasse, *Synthesis*, 396 (1981).

(B) 1-butanol/acetic acid/ethyl acetate/water, 1:1:1:1, (C) 1-butanol/acetic acid/water, 4:1:1, (D) 1-butanol/acetic acid/water, 4:1:5 (upper phase), (E) ethanol/water, 2:1, (F) 2-propanol/water, 7:3. Proton NMR spectra were recorded on Varian EM-390, Bruker WH-90, and Nicolet 250-MHz spectrometers. The mass spectral determinations of molecular weight were performed by Dr. Don Hunt and Jeff Shabanowitz at the University of Virginia using a triple quadrupole instrument modified for fast atom bombardment. HPLC was performed on a DuPont 850 liquid chromatography system. Zorbax C-8 and C-18 columns (4.6 mm × 250 mm) were employed for characterizing and assessing the purity of hydrogenolyzed product(s).

Solid-Phase Synthesis of [Asp^{25},Ala^{26},Gly^{27},Gln^{30}]-ACTH-(25–39)-OH: Synthesis of Boc-Asp(OBzl)-Ala-Gly-Glu(OBzl)-Asp(OBzl)-Gln-Ser(Bzl)-Ala-Glu(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-$OCH_2C_6H_4$-resin (1). Solid-phase synthesis was initiated with 25.0 g of Boc-Phe-OCH_2-C_6H_4-resin containing 0.77 mequiv of Boc-Phe per g of resin. The solid support consisted of 1% divinylbenzene–styrene copolymer. Initially the resin was swollen by stirring with toluene (500 mL) for 1 h, followed by deprotection via steps 1–9 as shown in Table I. Coupling of the subsequent N-terminal Boc-protected amino acids with appropriate side-chain protection, if required, was by the preformed hydroxybenzotriazole ester procedure, as per steps 10–13 of Table I.

The hydroxybenzotriazole active ester was prepared as in the following example: Boc-Glu(OBzl)-OH (13.5 g, 40 mmol) was dissolved in 1 M 1-hydroxybenzotriazole in DMF (40 mL) and toluene (150 mL) and chilled to 0–5 °C. DCC (2 M) in toluene (20 mL, 40 mmol) was added, and, after stirring for 30 min at room temperature, the precipitated dicyclohexylurea was removed by filtration and the filtrate added to the resin.

For coupling of the next amino acid, the resin was deprotected by following steps 1–9, and then leucine, residue 37, was introduced similarly. Starting with proline (residue 36), all of the first coupling steps were performed in DMF.

Whenever a second coupling was required, the resin was washed twice with methylene chloride (200 mL). The coupling was performed by using a symmetrical anhydride, 1.5 equiv, and the resin was washed as previously described. Second couplings were employed for Pro (36), Phe (35), Asp (29), Glu (28), and Asp (25).

The final weight of fully protected peptide resin was 60.0 g, which represents a weight increase of 35.0 g, suggesting a minimum of 90% of theoretical incorporation; with a second synthesis, performed similarly, starting with 26 g of Boc-Phe-$OCH_2C_6H_4$-resin, 61.1 g of compound 1 was obtained, corresponding to a weight increase of 35.1 g. Amino acid analysis of the peptide–resin was performed, after hydrolysis with 6 N HCl–propionic acid at 110 °C for 24 h, on a Dionex D-300 amino acid analyzer. The amino acid ratios are given in Table II; recovery was virtually quantitative with respect to the initial incorporation level (observed value was 0.76 mmol of peptide/g of resin).

Cleavage and Deprotection of Boc-Asp(OBzl)-Ala-Gly-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser(Bzl)-Ala-Glu-(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-$OCH_2C_6H_4$-resin (1). Method A. Hydrogen Fluoride Method.[26] Compound 1 (2.0 g) was treated with anhydrous hydrogen fluoride (10 mL) in the presence of *m*-cresol (1 mL) and 1,2-ethanedithiol (1 mL) as scavengers for carbocations liberated during acidolysis. The reaction mixture was gently stirred magnetically, maintaining a reaction temperature of 0 °C for 45 min. After removal of the hydrogen fluoride, the residue was triturated with ethyl acetate (40 mL). The insoluble material left behind was extracted with 1% aqueous acetic acid (50 mL) and filtered. Lyophilization of the filtrate afforded crude compound 4 (0.84 g, >99%; compound may contain non-peptide material). The dried insoluble resin residue weighed 0.73 g.

Method B. Solid-Phase Ammonium Formate CTH of Compound 1. A suspension of protected peptide resin 1 (760 mg) in 10 mL of dimethylformamide containing palladium(II) acetate (760 mg) was allowed to swell for 2 h under gentle stirring.[27] Next, a solution of ammonium formate (1 g) in water (1 mL) was added; following a brief induction period (~15 s), brisk evolution of gases (CO_2 and NH_3) with deposition of palladium onto the polystyrene beads occurred. After 2 h, the catalyst–resin was filtered and the resin washed with two 10-mL portions each of acetic acid and methanol. The filtrate and washes were combined and evaporated in vacuo to an oil. Upon addition of water (~20 mL) and potassium hydrogen sulfate (5 g), the Boc-protected pentadecapeptide precipitated. This was further washed with water (2 × 10 mL) and hexane (2 × 10 mL) and dried in vacuo to yield 285 mg of crude product. TLC analysis in solvent systems A–D indicated only two spots, the slower moving component being the major product; R_f(A) 0.72 and 0.66, R_f (B)0.63 and 0.53, R_f(C) 0.45 and 0.31, and R_f(D) 0.39 and 0.25. Quantitative amino acid analysis of protected peptide resin gave a value of 0.76 mequiv of peptide present per g of resin. The yield of the pentadecapeptide, assuming complete deblocking of all benzyl-based functionalities, was ~79%.

Debenzylation of the Residual Benzyl Ether on Serine: Solution-Phase Ammonium Formate CTH of Boc-Asp-Ala-Gly-Glu-Asp-Gln-Ser(Bzl)-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe-OH (2). The crude partially deblocked Boc-protected pentadecapeptide mixture of 2 and 3 (100 mg) was treated with ammonium formate (500 mg) and 10% palladium on carbon (200 mg) in 50% aqueous acetic acid (5 mL) for 1 h. TLC analysis indicated complete conversion of 2 to 3 [R_f(C) 0.32 for compound 3]. The catalyst was filtered through a plug of glass wool and Celite and washed with 1 × 10 mL each of 50% aqueous acetic acid and water. The combined filtrate and washes were adjusted to ~30% aqueous acetic acid solution by addition of water and lyophilized to constant weight. The yield of Boc-protected fully debenzylated peptide was 90 mg (95% of theory).

Method C. Ammonium Formate CTH of 1 in the Presence of Palladium on Resin and 20% Palladium Hydroxide on Carbon as the Catalysts: "One-Pot" Procedure. Palladium(II) acetate (2.0 g) was allowed to permeate into the peptide resin 1 (1.5 g) suspension in dimethylformamide (20 mL) for 2 h under gentle mixing on a magnetic stirrer. A solution of ammonium formate (2.0 g) in water (2 mL) was introduced into the reaction mixture. Within 30 s, brisk evolution of gases accompanied with deposition of palladium occurred. After 2 h, glacial acetic acid (18 mL) was added, followed by 20% palladium hydroxide on carbon (0.5 g), and the reaction was allowed to proceed for an additional 2 h. The catalyst was filtered and washed with 50% aqueous acetic acid (2 × 20 mL). The filtrate and washings were evaporated in vacuo in a Kugelrohr distillation apparatus, keeping the temperature below 40 °C. The residue was isolated by lyophilization from a 30% aqueous acetic acid solution to remove the volatile salts. The resulting solid was treated with a 50% solution of TFA in CH_2Cl_2 (40 mL) for 1 h. After evaporation of solvents, the resulting oil was dissolved in 30% aqueous acetic acid (6 mL) and applied to a Sephadex G-25 column (2.8 × 90 cm). Fractions of 6.8 mL were collected. The compound present in fractions 42–56 was pooled and lyophilized to yield Asp-Ala-Gly-Glu-Asp-Glu-Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe-OH as its acetate salt (0.67 g, overall yield is 94% of theory); R_f(E) 0.57 and R_f(F) 0.33.

Quantitative amino acid analysis determined the peptide content to be at least 94%. The product was identical in all respects including TLC and HPLC behavior with an authentic sample obtained by HF cleavage of peptide resin 1.

Identification of Boc-Asp-Ala-Gly-Glu-Asp-Gln-Ser-(Bzl)-Ala-Glu-Ala-Phe-Pro-Leu-Gly-Phe-OH (2) and Boc-Asp-Ala-Gly-Glu-Asp-Gln-Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe-OH (3) by FAB Mass Spectrometry and HPLC. The mixture of compounds 2 and 3 obtained by the solid-phase hydrogenolysis of peptide resin 1 was analyzed by fast-atom-bombardment mass spectrometry. Molecular ion peaks as positive ions at m/e 1820 and 1728, and as negative ions at m/e 1818 and 1726 for compounds 2 and 3, respectively, were observed. The difference in the molecular ion peaks corresponds to the presence of one benzyl moiety. Compound 3, obtained by the reduction

(26) S. Sakakibara, "Chemistry and Biochemistry of Amino Acids, Peptides and Proteins", Vol. 1, B. Weinstein, Ed., Marcel Dekker, New York, 1971, p 51.

(27) If solid palladium acetate is added, it is preferable to allow the resin–DMF suspension to stir with the catalyst for 8–12 h to ensure proper diffusion into the polymer matrix.

of compound 2, on independent mass spectrometric analysis furnished molecular ions as a positive ion at m/e 1728 and a negative ion at m/e 1726. The expected molecular ion peak for $C_{76}H_{108}N_{16}O_{30}$ (compound 3) is calculated as m/e 1725.82.

A linear gradient of acetonitrile (20–40% over 20 min) was run against 0.2 M triethylammonium phosphate buffer (pH 2.5) on a reverse-phase C-8 Zorbax analytical column (4.6 mm × 250 mm) maintained at 50 °C. The retention times (t_R) and k' values are summarized in Table III. Column eluent was detected at 214 nm. When a C-18 Zorbax analytical column (4.6 mm × 250 mm) with otherwise identical conditions was used, the retention times were somewhat longer. For example, compound 4 had a t_R of 9.84 min at a mobile-phase flow rate of 2 mL/min; the k' value was the same, within experimental error.

Registry No. 2, 86846-53-9; 3, 86853-41-0; 4, 86853-43-2; Boc-Glu(OBzl)-OH, 13574-13-5; ammonium formate, 540-69-2; ACTH, 9002-60-2.

Cyclic Sulfur Esters as Substrates for Nucleophilic Substitution. A New Synthesis of 2-Deoxy-2-fluoro-D-glucose

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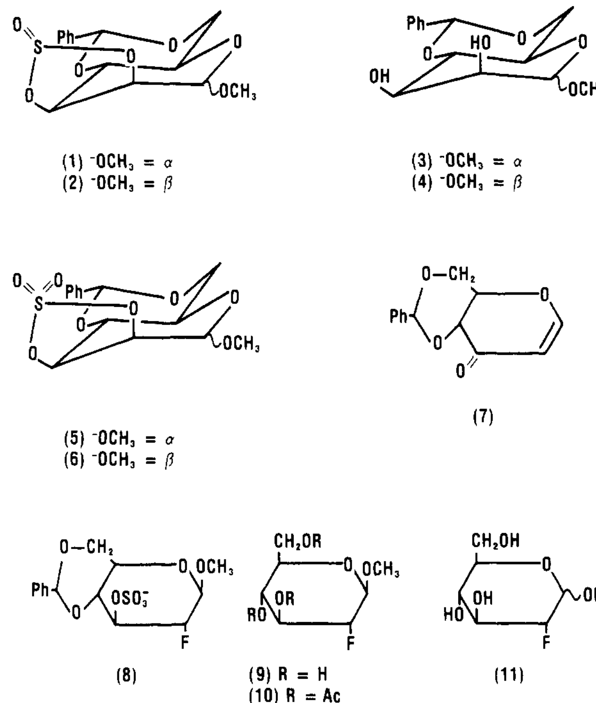
The 2,3-cyclic sulfites and sulfates of methyl 4,6-O-benzylidene-α- and -β-D-mannopyranoside were synthesized and examined as suitable substrates for the preparation of 2-deoxy-2-fluoro-D-glucose. Both sulfites on reaction with tetramethylammonium fluoride gave reaction products due to attack at sulfur by water. The methyl α-D-glycoside sulfate gave an α,β-unsaturated ketone (7). The β-methyl sulfate reacted cleanly with fluoride and other nucleophiles to give, after hydrolysis, the 2-substituted 2-deoxyglucose compounds.

2-Deoxy-2-fluoro-D-glucose-^{18}F has proved to be a useful radiopharmaceutical substance for studying glucose metabolism in man.[1,2] Because of the short half-life of fluorine-18 (110 min) both the radionuclide and the radiopharmaceutical compounds have to be freshly prepared for each study. However, the current synthesis, based upon F_2 addition to triacetal glucal,[3] is inefficient with respect to fluorine, which is the important criteria for evaluating yields with fluorine-18 reactions. Both 2-deoxy-2-fluoro-D-glucopyranosyl and -D-mannopyranosyl fluorides are formed and must be separated, and hydrolysis of the glycosyl fluoride leads to loss of half the remaining fluorine. As the production of fluorine-18 is very demanding on cyclotron time,[4] a synthesis of 2-deoxy-2-fluoro-D-glucose which can, in principal, utilize all the available fluorine-18 in the final product is desirable.

A synthetic route based upon fluoride ion displacement has the potential of utilizing all the available fluorine. High-yield reactions utilizing "no carrier added" fluorine-18 fluoride have been performed,[5–7] but nucleophilic displacement reactions at the 2-position of hexoses are difficult and normally give products other than those of direct displacement.[8]

Cyclic sulfur esters appeared to be attractive substrates to overcome these difficulties as the fully oxygenated sulfite or sulfate would be predicted to be a very good leaving group, and the transition states for alternative products would require highly strained tricyclic structures which should be unfavorable. However, after this work was completed the successful nucleophilic substitution of methyl 4,6-O-benzylidene-3-O-methyl-2-O-[(trifluoromethyl)sulfonyl]-β-D-mannopyranoside at the 2-position was reported.[9] It appears that the nature and stereo-

Chart I



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