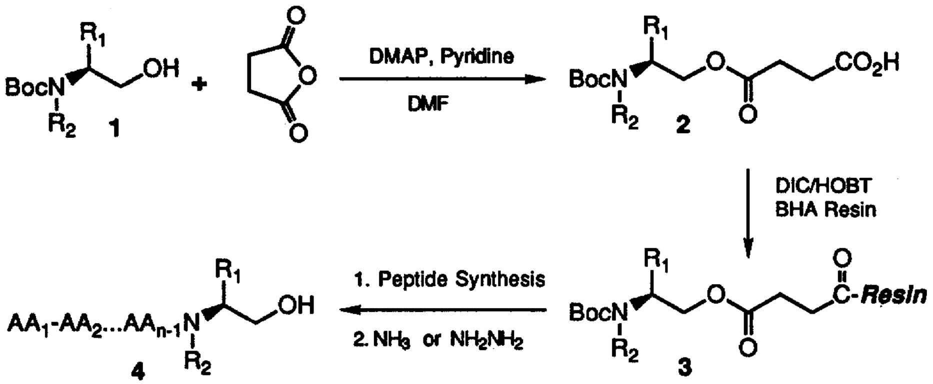
Tetrahedron Letters, Vol.30, No.38, pp 5045-5048, 1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Pergamon Press plc

A CONVENIENT PREPARATION OF C-TERMINAL PEPTIDE ALCOHOLS BY SOLID PHASE SYNTHESIS

J. Swistok, J. W. Tilley, W. Danho, R. Wagner*, K. Mulkerins
Roche Research Center, Hoffmann-La Roche, Inc., Nutley, NJ 07110

Summary: A new method is described which allows ready access to C-terminal peptide alcohols via solid phase synthesis. The procedure involves coupling of a hemisuccinate derived from a N-protected ß-aminoalcohol to a solid phase resin, elaboration of the desired peptide by conventional synthesis and cleavage of the resulting succinate with either ammonia or hydrazine.

Peptide alcohols occur in nature and are also of interest as analogs of bioactive peptides. These materials have generally been synthesized by hydride reduction of a peptide containing a C-terminal ester function,[1] or by a condensation strategy involving solution phase coupling of a preformed peptide with an amino alcohol.[2] There are two reported routes using solid phase methodology. They involve either ammonolysis of the resin bound peptide using the appropriate ß-aminoalcohol,[3] or lithium aluminum hydride reduction of the resin bound peptide ester at the completion of a conventional synthesis.[4] The limitations of these methods prompted us to consider an alternative approach in which an N-protected ß-aminoalcohol could be coupled to a solid support by esterification to a difunctional linking group, followed by conventional peptide synthesis and finally, ester cleavage to afford the target peptide alcohols.



We have found that the N-protected ß-aminoalcohols **1** react readily with succinic anhydride in the presence of 4-dimethylaminopyridine (DMAP) and pyridine in DMF to give the corresponding hemisuccinates **2** (Table 1). These monoacids were coupled to a benzhydrylamine resin (BHA) using diisopropylcarbodiimide DIC/HOBT, to provide the resin bound intermediates **3**, which can be further elaborated by conventional solid phase peptide synthesis. In order to illustrate this process, we have employed DIC/HOBT coupling of Boc protected aminoacids with TFA deprotections to construct a series of tetrapeptide derivatives. Liberation from the resin was accomplished by hydrolysis of the resulting succinic esters, either by treatment with ammonia/MeOH in a pressure bottle (72-96 h), or with an excess of hydrazine in DMF (24 h) to afford the tetrapeptide alcohols **4a** through **4g**. As indicated in Table 2, both hydrolysis methodologies provide comparable yields. The benzyl protecting group was removed from the tetrapeptide **4e** by simple Pd/C catalyzed hydrogenolysis in glacial acetic acid.

The method is compatible with either Boc/OFm and Fmoc/OtBu strategies, provided that the timing of side chain deprotection is appropriate, and provides an exceptionally facile route to peptide alcohols. The application of this technology to the construction of biologically active peptide alcohols will be reported in due course.

Table 1[5] ß-Aminoalcohol-hemisuccinnates

Compd	R_1	R_2	% Yield	$[\alpha]_D$[a]	Recryst. Solv.	mp (°C)
2a	H	H	62		Et_2O/hexanes	84-85.5
2b	(S)-2-butyl	H	75	-16.42°	Et_2O/hexanes	90-92
2c	$C_6H_{11}CH_2$	H	59	-18.75°	EtOAc/hexanes	87-89
2d	$C_6H_5CH_2$	H	85	-24.34°	EtOAc/hexanes	94.5-95.5
2e	$C_6H_5CH_2OCH_2$	H	44	+ 6.03°	Toluene/hexanes	90-92.5
2f	$CH_3SCH_2CH_2$	H	75	-20.86°	Et_2O/hexanes	81.5-83
2g	$-CH_2CH_2CH_2-$		93	-41.75°		oil

a. rotations were run at 25° C in ethanol (c = 1) except for **2d** ($CHCl_3$, c = 0.55).

Table 2 Yield of Tetrapeptide Alcohols

Compd		Hydrolysis (% Yield)[8,9]	
		NH_3	N_2H_4
4a	LeuAlaGlyVal-Glycinol	14	17
4b	LeuAlaGlyVal-Isoleucinol	28	41
4c	LeuAlaGlyVal-Cyclohexylalaninol	50	62
4d	LeuAlaGlyVal-Phenylalaninol	78	78
4e	LeuAlaGlyVal-L-Serinol(Bzl)	85	77
4f	LeuAlaGlyVal-Methioninol	44	38
4g	LeuAlaGlyVal-Prolinol	77	79

General Procedure for the synthesis of **2**:

At room temperature, compound **1** (10 mmol), succinic anhydride (3.0 g, 30 mmol, 3 eq.), DMAP (3.7 g, 30 mmol), and pyridine (3.8 mL) were stirred in 25 mL DMF (12 h). After removal of the majority of DMF under reduced pressure, the residue was dissolved in water (100 mL) and the pH brought to 9.0 using conc. NH_4OH. The mixture was washed with EtOAc (2 x 20 mL). The aqueous layer was carefully acidified to pH 3.0 using conc. HCl and then extracted with EtOAc (3 x 30 mL). Combination of the organic extracts was followed by washing with water (3 x 30 mL) and brine (30 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to constant weight (0.01 torr). The crude products obtained in this way are suitable for use in the next step.

General Procedure for the synthesis of **4**:

The succinates **2** (2.05 mmol) were coupled to the BHA resin[6] (2.0 g, 0.82 mmol) using DIC (310 µL, 2.05 mmol) and HOBT (390 mg, 2.05 mmol)) in DMF (40 mL). The mixture was agitated until a negative ninhydrin test was obtained. All subsequent, deprotections, washings and couplings proceeded in the conventional manner.[7]

Ammonolysis

Ammonia (40 mL) was condensed into a chilled (-40 °C) pressure bottle containing the peptide-resin (1.5 g) and methanol (60 mL). The vessel was sealed and allowed to stir (72-96 h). After chilling the pressure bottle (-40 °C), the vessel was opened and the NH_3 allowed to evaporate. The methanolic solution was then filtered and the remaining resin was washed with methanol. The filtrate was concentrated in vacuo and the resulting crude residue dissolved in acetic acid and lyophilized. The crude product was redissolved in AcOH/H_2O (6 mL, 5:1) and purified by reverse phase HPLC on a Whatman C_{18} Mag 40 (40 mm x 50 cm) column using gradient elution 5% CH_3CN/0.025% TFA to 30% CH_3CN/0.025% TFA. The desired fractions were pooled and lyophilized. Yields for the various peptide alcohols are given in Table **2**.

Hydrazinolysis

The peptide resin (1.5 g) was treated with hydrazine (4.0 mL) in DMF (25 mL) with shaking (24 h). The mixture was filtered and the remaining resin was washed with DMF (2 x 25 mL). The combined filtrates were then concentrated under high vacuum (0.1 torr, bath temp 45 °C) to constant weight and sufficient Et_2O was added to the oily mass to precipitate crude solid product. The product was dissolved in AcOH/H_2O (6 mL, 5:1) and purified by reverse phase HPLC as before. Yields for the various peptide alcohols are given in Table **2**.

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4. Stewart, J. M.; Morris, D. H. Pennwalt Corporation, US Patent 4254023 (1981); Stewart, J. M.; Morris, D. H.; Chipkin, R. E. Pennwalt Corporation, US Patent 4254024 (1981).
5. All compounds have been characterized by combustion analyses, IR, ^{1}H NMR, and mass spectra. With the exception of **2g**, every compound possesses an elemental analysis within the error limits of 0.4%. Anal. Calcd for $C_{14}H_{23}N_1O_6$ (**2g**): C, 55.80; H, 7.69; N, 4.65. Found: C, 54.18; H, 7.58; N, 4.22; H_2O content by Karl Fisher analysis, 0.39%.
6. Polystyrene crosslinked with 1% divinylbenzene, 0.41 mmol/g.
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8. Yields based on **3**.
9. All final peptide products were characterized by amino acid and mass spectral analysis (FAB).

Acknowledgments: The authors thank members of the Physical Chemistry Department, Hoffmann-La Roche, Inc. for determination of the spectral and microanalytical data for the compounds reported herein. We also thank Drs. R. W. Kierstead and A. M. Felix for their support and advice during the course of these studies.

(Received in USA 14 June 1989)