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HYPERSENSITIVE ACID-LABILE (HAL) TRIS(ALKOXY)BENZYL ESTER ANCHORING FOR SOLID-PHASE SYNTHESIS OF PROTECTED PEPTIDE SEGMENTS[1,2]

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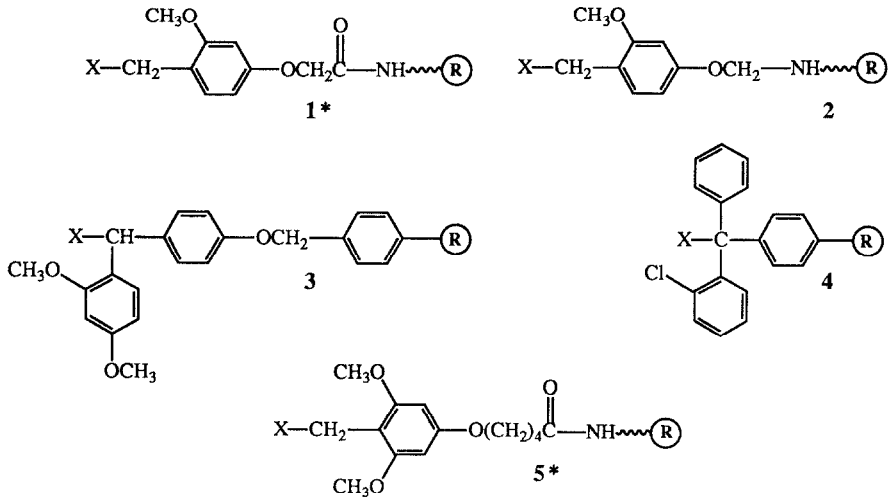
Summary: The novel 5-(4-hydroxymethyl-3,5-dimethoxyphenoxy)valeric acid (HAL) handle has been prepared and applied to solid-phase peptide synthesis using 9-fluorenylmethyloxycarbonyl (Fmoc) for N^{α}-amino protection and *tert*-butyl ethers, esters, and urethanes for side-chain protection. Otherwise fully protected peptide *acids*, which are needed for segment condensation studies, were obtained in excellent yields and purities by cleavage of the resultant tris(alkoxy)benzyl ester anchoring linkage with appropriate cocktails containing 0.05 - 0.1% (v/v) trifluoroacetic acid.

Partially protected peptide segments are required intermediates for the preparation of larger peptides or small proteins by segment condensation approaches either in solution or on a polymeric support.[3,4] Solid-phase synthesis[4] is widely acknowledged to offer the best prospects for rapid and efficient assembly of peptide chains, but until relatively recently, the needed levels of selectivity in conditions for N^{α}-amino and side-chain deprotection, and in procedures for detachment of peptides from the support, have not been available. Assuming that a peptide can be cleaved successfully to furnish a free C^{α}-carboxyl group with all other functional groups remaining protected, the resultant intermediate can be purified before its further use in segment condensation.[5]

In recent years, a strategy employing the *orthogonal*[6] combination of base-labile 9-fluorenylmethyloxycarbonyl (Fmoc) for N^{α}-amino protection and acid-labile *tert*-butyl derivatives for side-chain protection has gained considerable popularity,[4b,7] because it avoids the relatively harsh final cleavage conditions of the more conventional strategy[4] based on the graduated lability to acid of *tert*-butyloxycarbonyl (Boc) for N^{α}-amino protection and benzyl or cyclohexyl derivatives for side-chain protection. In the Fmoc/*t*Bu strategy, a third dimension of orthogonality can be provided by use of *ortho*-nitrobenzyl (photolabile),[5f,7a] silicon-containing (fluoride-labile),[8] or allyl-derived [cleaved with Pd(0)][9] anchoring linkages. While highly elegant, the aforementioned orthogonally cleavable anchors are prepared by multi-step routes, and in some cases, the applications to solid-phase synthesis of protected peptide segments do not scale up well.

Consequently, it becomes attractive to pursue the alternative approach of using anchoring linkages (Scheme 1) that are cleaved with extremely dilute acid. The latter strategy requires exquisite "fine-tuning" of the anchor structure and the corresponding removal conditions. The (4-hydroxymethyl-3-methoxyphenoxy)acetic acid handle of Atherton, Sheppard, and Williams,[7a,10] and the closely related 2-methoxy-4-alkoxybenzyl alcohol (SASRIN) support of Mergler *et. al.*[11] lead to bis(alkoxy)benzyl ester anchoring linkages (**1b** and **2b**) which cleave with 1% (v/v) trifluoroacetic acid (TFA) in dichloromethane. The cleavage conditions promote premature side-chain deprotection at Lys(Boc) and Tyr(*t*Bu).[10] On the other hand, the trialkoxydiphenylmethyl ester resin of

Scheme 1. Anchoring Linkages Suitable for Release of Peptide Carboxyls with Dilute Acid*



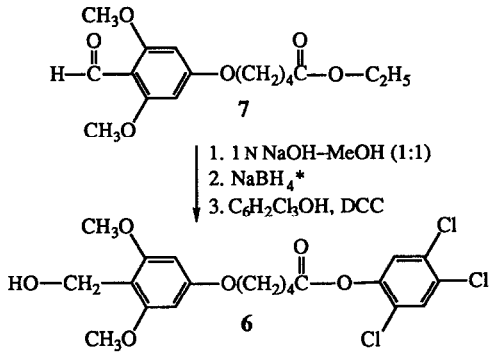
a X = OH (structures **1** - **3**, and **5**), X = Cl (structure **4**)
b X = RCO_2-, α-carboxylate function of protected amino acid or peptide

*Structures **2a**, **3a**, and **4a** are derivatives of polystyrene-resins, and are further converted to the ester derivatives (series **b**). Structures **1a** (ref. 10) and **5a** (this work) are obtained by amide bond formation between the activated carboxyl function of an appropriate handle and the amino group of a suitably functionalized support (see also ref. 1). Since amino groups can be introduced onto a wide range of polymeric materials in addition to polystyrene, including polyamides, silica, and polyethylene glycol-polystyrene grafts, the handle approach allows considerable versatility for the critical beginning step of solid-phase synthesis.

Rink[12] or the *ortho*-chlorotrityl resin of Barlos *et al.*[13] lead to esters (**3b** and **4b**) which cleave with 10% (v/v) acetic acid in dichloromethane, but also cleave prematurely in the presence of a free C^{α}-carboxyl group of an incoming protected amino acid during each coupling step.

The present paper reports that a novel tris(alkoxy)benzyl ester (HAL)[14] anchor (**5b**) has the optimal balance of acid-lability properties. The key handle intermediate for this work, 2,4,5-trichlorophenyl 5-(4-hydroxymethyl-3,5-dimethoxyphenoxy)valerate (**6**) was obtained in 59% yield by a three-step one-pot procedure (Scheme 2) starting from intermediate **7** already available in our laboratory from studies on a related handle.[14, 15b] Handle **6** (2 equiv.) was attached quantitatively *via* its pre-activated carboxyl group to amino-functionalized supports (1 equiv.) by 2 h couplings at 25 °C in the presence of 1-hydroxybenzotriazole (HOBt; 2 equiv.), using *N,N*-dimethylformamide (DMF) as solvent. Next, the *C*-terminal protected Fmoc-amino acid derivative (5 equiv.) could be anchored to the nucleophilic hydroxymethyl group of the resin-bound handle, in 85-95% yield, by *N,N*-diisopropylcarbodiimide (DIPCDI; 5 equiv.)-mediated coupling, catalyzed by 4-dimethylaminopyridine (DMAP; 0.5 equiv.), 1 h, 25 °C, with DMF as solvent.[16] Stepwise deprotection/coupling cycles to incorporate subsequent amino acid residues proceeded well by standard Fmoc protocols used in our laboratory.[15]

Scheme 2



*The resultant benzyl alcohol with a valeric acid side-chain was unstable; consequently it was extracted into ethyl acetate and carried forward directly through the 2,4,5-trichlorophenol esterification step.

The HAL linkage (**5b**) proved to be stable (>95% retention of chains from Fmoc-Gly-Val-Ala-O-HAL-Ⓡ after 24 h, 25 °C) to HOBt (0.1 M)–DMF and Boc-amino acids (0.1 M)–DMF. However, HAL was labile to 10% (v/v) acetic acid in dichloromethane (78% cleaved in 24 h; compare to reported properties of linkages **3b** and **4b**, which are *more* sensitive to acid). For convenient cleavage of HAL, very dilute solutions of trifluoroacetic acid (TFA) in dichloromethane were used. The model tripeptide linked to HAL was cleaved completely after 5 min with 0.1% (v/v) TFA or after 45 min with 0.05% (v/v) TFA, whereas 0.01% (v/v) TFA gave 80% cleavage in 3 h.

Demonstrations of the usefulness of HAL anchoring were provided by the syntheses of several protected segments[17] with sequences related to human gastrin-I. Fmoc-[Glu(O*t*Bu)]$_5$-Ala-OH, Fmoc-[Tyr(*t*Bu)]$_5$-Ala-OH, and Fmoc-[Lys(Boc)]$_5$-Ala-OH were each obtained in >97% purity (HPLC) after essentially quantitative cleavage with 0.1% (v/v) TFA in CH_2Cl_2, for 1 h at 25 °C. The pure tryptophan-containing peptide[18] pGlu-Gly-Pro-Trp-Leu-OH was obtained in 70-76% (v/v) yield, upon cleavage with 0.05% (v/v) TFA in CH_2Cl_2–β-mercaptoethanol–anisole (97:2:1) or 0.05% (v/v) TFA in CH_2Cl_2–thioanisole–1,2–ethanedithiol–anisole (90:5:3:2), 25 °C, 1 h. Omission of scavengers approximately halved the yield, indicating tryptophan alkylation/back-addition to support. Alternatively, an increased concentration of TFA did not improve the cleavage yield, either with or without scavengers.

In conclusion, the new handle **6** is conveniently made (Scheme 2) and can be used with any amino-functionalized support. A tris(alkoxybenzyl) ester **5b** anchor is obtained that has acid sensitivity/stability intermediate to anchors **1b** - **4b** described by others (Scheme 1). Consequently, the anchor remains entirely intact throughout the assembly of peptide chains by Fmoc chemistry, but final cleavage with cocktails containing very dilute TFA provides in high yields and purities protected peptide acids, without premature removal of *tert*-butyl-based side-chain protecting groups.[19]

Acknowledgments

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References and Notes

1. A preliminary report of this work on the HAL handle was included in the authors' contribution to the 21st European Peptide Symposium, September 2-8, 1990, Platja d'Aro, Spain. At the same meeting, A. Flörsheimer and B. Riniker reported on the use of the related [one fewer aryl methoxy group, one less methyene group in the spacer] 4-(4-hydroxymethyl-3-methoxyphenoxy)butyric acid (HMPB) handle. Both articles will appear in the published proceedings of the meeting, Escom, Leiden, 1991.
2. Abbreviations used are: Boc, *tert*-butyloxycarbonyl; DIPCDI, *N,N*-diisopropylcarbodiimide; DMAP, 4-dimethylaminopyridine; DMF, *N,N*-dimethylformamide; Fmoc, 9-fluorenylmethyloxycarbonyl; HAL, 5-(4-hydroxymethyl-3,5-dimethoxyphenoxy)valeric acid handle; HOBt, 1-hydroxybenzotriazole; PAL, 5-(4-(9-fluorenylmethyloxycarbonyl)aminomethyl-3,5-dimethoxyphenoxy)valeric acid handle; SASRIN, 2-methoxy-4-alkoxybenzyl alcohol resin; TFA, trifluoroacetic acid.
3. Review: F.M. Finn and K. Hofmann in *The Proteins* (H. Neurath and R.L. Hill, eds.), 3rd Ed., Volume 2, Academic Press, New York, 1976, pp. 105-253.
4. Reviews: (a) G. Barany and R.B. Merrifield in *The Peptides* (E. Gross and J. Meienhofer, eds.), Volume 2, Academic Press, New York, 1979, pp. 1-284; (b) G. Barany, N. Kneib-Cordonier and D.G. Mullen, *Int. J. Peptide Protein Res.* **30**, 705-739 (1987).
5. For recent examples from our laboratories and others, see: (a) T. Kubiak, D.B. Whitney, and R.B. Merrifield, *Biochemistry* **26**, 7849-7855 (1987), and references cited therein; (b) T.A. Lyle, S.F. Brady, T.M. Ciccarone, C.D. Colton, W.J. Paleveda, D.F. Veber, and R.F. Nutt, *J. Org. Chem.* **52**, 3752-3759 (1987); (c) E.T. Kaiser, H. Mihara, G.A. Laforet, J.W. Kelly, L. Walters, M.A. Findeis, and T. Sasaki, *Science* **243**, 187-192 (1989); (d) F. Albericio, M. Pons, E. Pedroso, and E. Giralt, *J. Org. Chem.* **54**, 360-366 (1989); (e) A. Grandas, F. Albericio, J. Josa, E. Giralt, E. Pedroso, J.M. Sabatier, and J. van Rietschoten, *Tetrahedron* **45**, 4637-4648 (1989); (f) N. Kneib-Cordonier, F. Albericio and G. Barany, *Int. J. Peptide Protein Res.* **35**, 527-538 (1990).
6. An *orthogonal* system is defined as one using two or more independent classes of protecting groups that are removable by differing chemical mechanisms. See ref. 4a, p. 12, and G. Barany and R.B. Merrifield, *J. Am. Chem. Soc.* **99**, 7363-7365 (1977).
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9. (a) H. Kunz and B. Dombo, *Angew. Chem. Int. Ed. Engl.* **27**, 711-713 (1988); (b) B. Blankemeyer-Menge and R. Frank, *Tetrahedron Lett.* **29**, 5871-5874 (1988).
10. R.C. Sheppard and B.J. Williams, *J. Chem. Soc., Chem. Commun.*, pp. 587-589 (1982).
11. M. Mergler, R. Nyfeler, R. Tanner, J. Gosteli, and P. Grogg, *Tetrahedron Lett.* **29**, 4009-4012 (1988).
12. H. Rink, *Tetrahedron Lett.* **28**, 3787-3790 (1987).
13. K. Barlos, D. Gatos, J. Kallitsis, G. Papaphotiu, P. Sotiriu, Y. Wenqing, and W. Schäfer, *Tetrahedron Lett.* **30**, 3943-3946 (1989).
14. The acronym "HAL" derives from *h*ypersensitive *a*cid-labile *l*inker, and emphasizes the structural similarity to "PAL" (see refs. 2 and 15), a handle used to prepare peptide amides with Fmoc chemistry.
15. (a) F. Albericio, and G. Barany, *Int. J. Peptide Protein Res.* **30**, 206-216 (1987); (b) F. Albericio, N. Kneib-Cordonier, S. Biancalana, L. Gera, I. Maseda, D. Hudson, and G. Barany, *J. Org. Chem.* **55**, 3730-3743 (1990), and references cited in both of these papers.
16. Based on an assay described by X. Jorba, F. Albericio, A. Grandas, W. Bannwarth, and E. Giralt, *Tetrahedron Lett.* **31**, 1915-1918 (1990), the level of racemization for this esterification was judged to be <0.5%.
17. All protected peptides prepared in this work gave satisfactory ratios of amino acids upon hydrolysis, and showed the expected ions upon fast atom bombardment mass spectrometry. See ref. 5f for a description of the syntheses of authentic Fmoc-[Glu(O*t*Bu)]$_5$-Ala-OH (on a photo-labile support) and pGlu-Gly-Pro-Trp-Leu-OH (on a *p*-alkoxybenzyl support). Both of the syntheses from ref. 5f required careful optimization of cleavage conditions to minimize certain side reactions; the procedure reported herein with HAL gave better yields and purities.
18. Tryptophan alkylation occurs under acidic conditions when using supports that give rise to stable carbonium ions. See refs. 5f and 15b, and literature cited therein.
19. After cleavage of peptide-resins, the combined filtrates and washings should be diluted with water prior to evaporation of TFA and organic solvents. Peptide products are obtained after lyophilization.

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