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A NEW FLUORIDOLYSABLE ANCHORING LINKAGE FOR ORTHOGONAL SOLID-PHASE PEPTIDE SYNTHESIS: PREPARATION AND PROPERTIES OF THE N-(3 OR 4)-[[[(4-HYDROXYMETHYL)-PHENOXY-t-BUTYLPHENYL]SILYL]PHENYL]PENTANEDIOIC ACID, MONOAMIDE (PBS) HANDLE[1-3]

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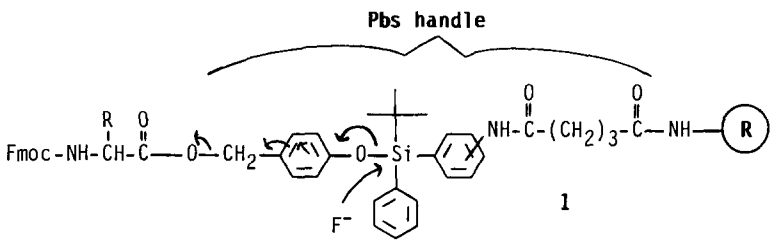
The synthesis and characterization of a new silicon-containing handle for use in solid-phase peptide synthesis is described. The anchoring linkage derived from this new handle, when treated with fluoride (1.0 equiv.) for 5 min at 25 °C, releases peptides as their free acids in essentially quantitative yields and without racemization.

As part of a program[4] to develop mild chemical methods for solid-phase peptide synthesis[5], we require new orthogonally[6] cleavable handles[7,8] for attaching chains to the support. Silyl ether-containing linkages were considered as attractive candidates because of the possibility for facile cleavage under neutral conditions of fluoridolysis[9,10]. We report here the preparation of the new protected N-(3 or 4)-[[4-(aminoacyloxymethyl)phenoxy-t-butylphenyl]silyl]phenyl-N'-resinylpentanedioic acid, diamide (1) which is designed so



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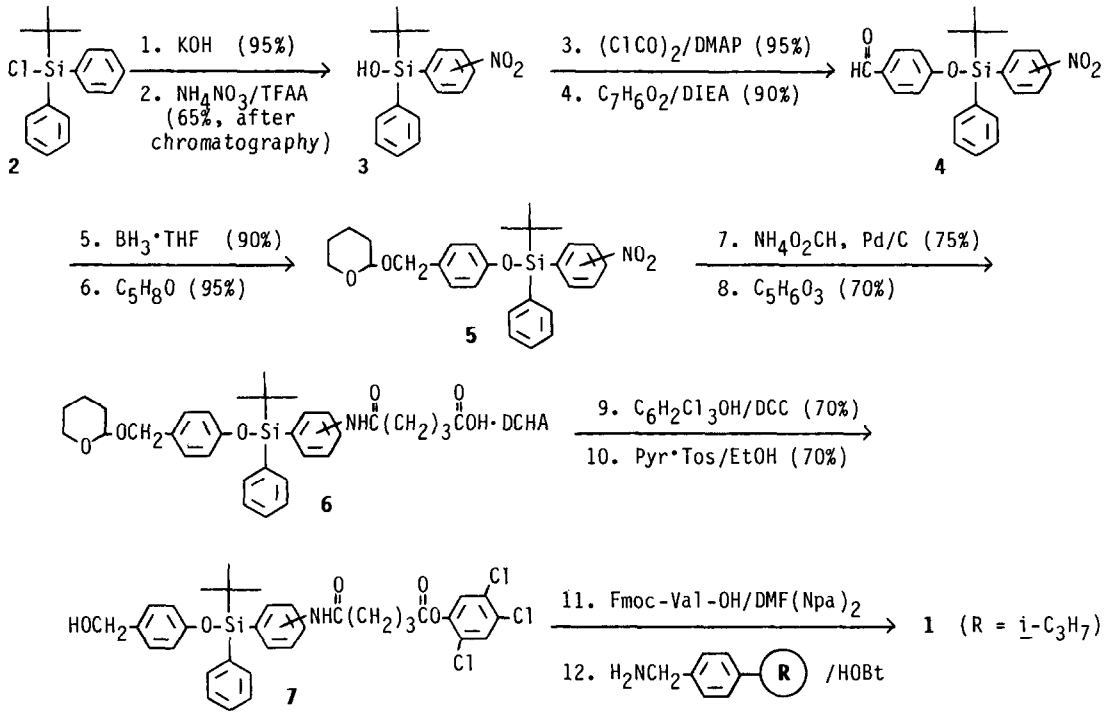
that rapid silicon-oxygen[9] bond fission with one equivalent of fluoride at 25 °C is followed directly by a 1,6-elimination mechanism[11] leading to a free peptide acid (see above diagram). We further show how this anchoring linkage, which because of the p-alkoxybenzyl ester[12] moiety can also be cleaved by trifluoroacetic acid at 25 °C, is compatibly applied with N^{α}-9-fluorenylmethyloxycarbonyl (Fmoc)[13] and N^{α}-dithiasuccinoyl (Dts)[4] amino acids for stepwise solid-phase synthesis of a simple model peptide.

Resin derivative **1** was synthesized in twelve steps (see Scheme)[14] starting from commercially available t-butylchlorodiphenylsilane (**2**). Chlorosilane **2** could be cleanly hydrolysed [KOH (1.1 equiv.) in H_2O—MeOH—Et_2O (4:1:6), 25 °C, 12 h] to the relatively inert corresponding silanol, which was carefully nitrated (-23 °C, 5 h) with ammonium nitrate (1.15 equiv.) according to Crivello[15]. The desired mononitrosilanol 3 was isolated

apart from unreacted starting material and the dinitro compound by silica gel chromatography [Et_2O—hexane (3:17)]. It was established by two dimensional COSY NMR at 300 MHz that the meta and para substituted isomers both formed (4:1 ratio). This ratio of isomers carried over for subsequent products. Next, a new method was developed using oxalyl chloride (4 equiv.) and DMAP (1.5 equiv.) in CH_2Cl_2 (25 °C, 36 h) to transform the silanol back to a chlorosilane. Subsequent reaction (12 h, under N_2) with p-hydroxybenzaldehyde (1.1 equiv.) in the presence of N,N-diisopropylethylamine (1.5 equiv.) in dry CH_2Cl_2 gave silyl ether **4**.

Scheme[3,14]



The aldehyde of **4** was then reduced (under N_2) using borane-THF complex (1.0 equiv.) in ethyl ether (25 °C, 1 h) to provide a benzylic alcohol function which was protected[16] as the tetrahydropyranyl ether. These steps gave **5**, which required modification before attachment to polymeric supports was possible. Consequently, the nitro group of **5** was reduced[17] by catalytic transfer hydrogenation [NH_4O_2CH (9.0 equiv.), Pd/C] in methanol (25 °C, 2 h) to give an amine, which was then acylated with glutaric anhydride[18] (1.1 equiv.) in chloroform (25 °C, 12 h). The product of these steps was isolated as the solid dicyclohexylammonium salt **6**, mp 101 °C, and could be converted back to the free acid by neutralization with Dowex-50 ion-exchange resin. The acid was then esterified with 2,4,5-trichlorophenol (1.1 equiv.) using as the coupling agent N,N'-dicyclohexylcarbodiimide in CH_2Cl_2 (25 °C, 12 h), and finally deprotection with pyridinium tosylate[16] (0.1 equiv.) in ethanol (50 °C, 4 h) gave the new silicon-functionalized handle **7**. The "preformed handle" approach[4b,8a,d,e

for attachment of amino acids to the polymeric support was then applied; thus, suitably protected amino acids were esterified to **7** using N,N-dimethylformamide dineopentyl acetal[8d] as the condensing agent. The amino acyl handle (3.0 equiv.) was then quantitatively attached onto an aminomethylcopoly(styrene-1%-divinylbenzene) resin (0.19 meq/gm) by overnight reaction (25 °C) in the presence of 1-hydroxybenzotriazole (3.0 equiv.) in N,N-dimethylformamide.

Resin **1** was the starting point for several syntheses of the model tetrapeptide L-leucyl-L-alanylglycyl-L-valine. Both Fmoc and Dts-amino acids were incorporated under standard conditions of this laboratory[4b,8d] for DCC-mediated coupling. Upon completion of chain assembly, the peptide was rapidly cleaved by tetrabutylammonium fluoride (1.0 equiv.) in N,N-dimethylformamide at 25 °C in 5 min. The cleavage yields were ≥97% based on amino acid remaining on the resin, and the purities were ≥98% as judged by ion-exchange chromatography. In one synthesis, the last amino acid was introduced with t-butyloxycarbonyl (Boc) for N^{α}-amino protection, and the orthogonal fluoridolysis conditions released the protected peptide acid Boc-Leu-Ala-Gly-Val-OH[19,20]. Lastly, the issue of racemization was addressed by applying the Manning-Moore assay[21] on the dipeptide alanylvaline sequence assembled on the resin and cleaved by fluoridolysis. Only the L,L-diastereomer was observed (sensitivity limit 0.05%).

References and Notes

1. A preliminary report on this topic was presented by D.G.M. at the 187th National American Chemical Society Meeting in St. Louis, MO , April 8-13, 1984. Recently, we learned of independent work carried out by R. Ramage and colleagues at the University of Edinburgh, Scotland and reported at the Nineteenth European Peptide Symposium, Porto Carras-Chalkidiki, Greece, August 31-September 5, 1986. These workers prepared a resin-bound variant of the Tmse group (see ref. 10a) and used it to prepare a model peptide which was cleaved by fluoridolysis.

2. Financial support from the National Institutes of Health (GM 28934 and AM 01099) is gratefully acknowledged. D.G.M. thanks the Henkel Corporation for a graduate fellowship, 1984-1985.

3. Abbreviations used are: Boc, t-butyloxycarbonyl; DCC, N,N'-dicyclohexylcarbodiimide; DCHA, N,N-dicyclohexylamine; DIEA, N,N-diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMF, N,N-dimethylformamide; DMF(Npa)$_2$, N,N-dimethylformamide dineopentyl acetal; Dts, dithiasuccinoyl; Fmoc, 9-fluorenylmethyloxycarbonyl; HOBt, 1-hydroxybenzotriazole; Pbs, title handle of this paper; Pyr$^+$Tos, pyridinium tosylate; (R), polystyrene resin support; TFAA, trifluoroacetic anhydride; THF, tetrahydrofuran; Tmse, 2-trimethylsilylethyl

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6. An orthogonal system is defined as one using two or more independent classes of protecting groups that are removable by differing chemical mechanisms. Consequently, these groups can be selectively removed in any order in the presence of the other classes, see refs 4a, 5b.

7. A handle is defined as a bifunctional spacer that serves to link the first amino acid to the resin in two discrete steps, see refs 5b and 5c for reviews and additional examples.

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