N^{ϵ}-[[2-(Trimethylsilyl)ethoxy]carbonyl] Derivatives of Tri-L-lysine and Tetra-L-lysine as Potential Intermediates in the Block Polymer Synthesis of Macromolecular Drug Conjugates

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Tri-L-lysine and tetra-L-lysine derivatives were synthesized with N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl] (N^{ϵ}-Teoc) protecting groups on all the lysines, or on all but the N-terminal lysine, and with N^{α}-(*tert*-butyloxycarbonyl) (N^{α}-Boc) or N^{α}-(9-fluorenylmethyloxycarbonyl) (N^{α}-Fmoc) groups on the N-terminal lysines. Treatment of the Boc/Teoc peptides with *p*-toluenesulfonic or 2,4,6-trimethylbenzenesulfonic acid led to Boc cleavage with Teoc retention only when the Teoc/Boc ratio was 1:1 or 2:1. In contrast, treatment of the Fmoc/Teoc peptides with liquid ammonia in a sealed vessel cleaved the Fmoc group without significant loss of Teoc groups even when the Fmoc/Teoc ratio was 3:1, showing that Fmoc and Teoc groups provide more selectivity than the Boc and Teoc combination. N^{α}-Fmoc and N^{ϵ}-Teoc groups were both stable under catalytic hydrogenolysis conditions. This made it possible to prepare N^{α}-Fmoc-tri-L-lysine and N^{α}-Fmoc-tetra-L-lysine derivatives with N^{ϵ}-Teoc groups on all but the N-terminal lysine and demonstrated that the triad Fmoc/Cbz/Teoc is superior to Boc/Cbz/Teoc in peptide synthesis involving the orthogonal protection strategy.

Cytotoxicity and selectivity are dominant themes throughout the history of cancer chemotherapy.[1] One strategy for improving the selectivity of anticancer agents is to link them to a polymer. Attachment to the polymer prevents uptake by diffusion or channel-mediated transport but allows uptake by endocytosis, which is extremely efficient in tumor cells.[2-4] Within the cells, the activity of some of the 40 known lysosomal enzymes may then release the drug from the carrier, restoring cytotoxicity.[3] A macromolecule possessing many favorable characteristics for selective drug delivery by this approach is poly(L-lysine),[5] which is rapidly taken up and hydrolyzed within tumor cells. In contrast, poly(D-lysine) is taken up rapidly but is not degraded, making it possible to compare the biological activities of drugs conjugated to degradable versus nondegradable polymers. Poly(L-lysine) has been used previously as a carrier for the well-known anticancer drug methotrexate (4-amino-4-deoxy-10-(methylpteroyl)-L-glutamic acid, MTX).[6-10] A noteworthy feature of these MTX–poly(L-lysine) conjugates is that they are inhibitors of the growth of tumor cells that are resistant to MTX by virtue of a defect in the normal carrier-mediated active transport pathway for this drug.

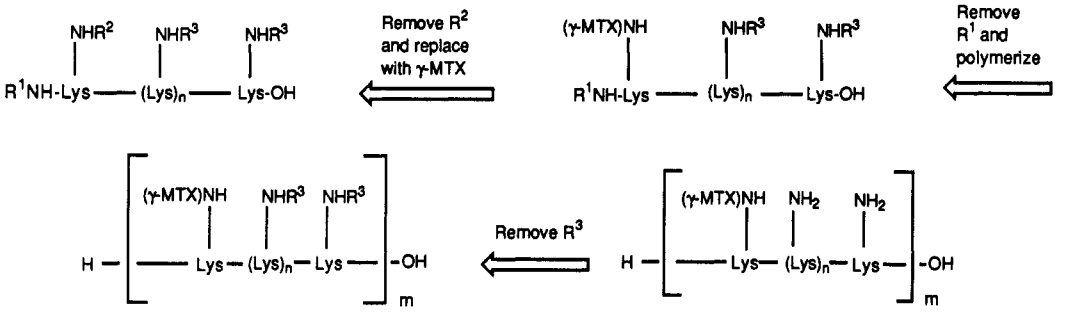
Most biological studies on drug conjugates to poly(L-lysine) have utilized random coupling to unspecified ϵ-amino groups. In the preparation of MTX–poly(L-lysine) derivatives, the coupling reactions have, with one exception,[10] been nonregiospecific, giving adducts in which the polymer is linked to either or both of the α- and γ-carboxyl groups in the MTX moiety. Bifunctional activation of these carboxyls can also result in cross-linking of carrier chains. The resulting heterogeneity complicates the interpretation of biological data and disallows fine-tuning of the cleavage kinetics. By attaching the ligands at regularly spaced intervals along the polymer backbone, a range of ligand release rates can be achieved and the most effective structures selected.[11] In this context we have been interested in precursors that provide specific sites for ligand attachment. In previous papers we reported the synthesis of regiospecific, non-cross-linked MTX(γ–ϵ)-poly-L-lysine adducts,[12] and of protected[13] as well as nonprotected[14] di- and trilysine adducts of MTX,[13,14] the latter of which may be viewed as likely products of the lysosomal hydrolysis of MTX(γ–ϵ)poly-L-lysine. In this paper we report methods of preparation of protected di-, tri-, and tetra-L-lysines that can be used to generate drug–poly(L-lysine) conjugates of defined sequence by block polymerization.[15] By this means, one might be able, for example, to use a protected MTX(γ–ϵ)tetra-L-lysine to prepare MTX(γ–ϵ)poly-L-lysine with a pendant γ-MTX moiety on every fifth lysine residue.

Block polymerization as a route to poly(L-lysine) drug conjugates poses a challenge, in that it requires the use of three different and mutually compatible N-protecting

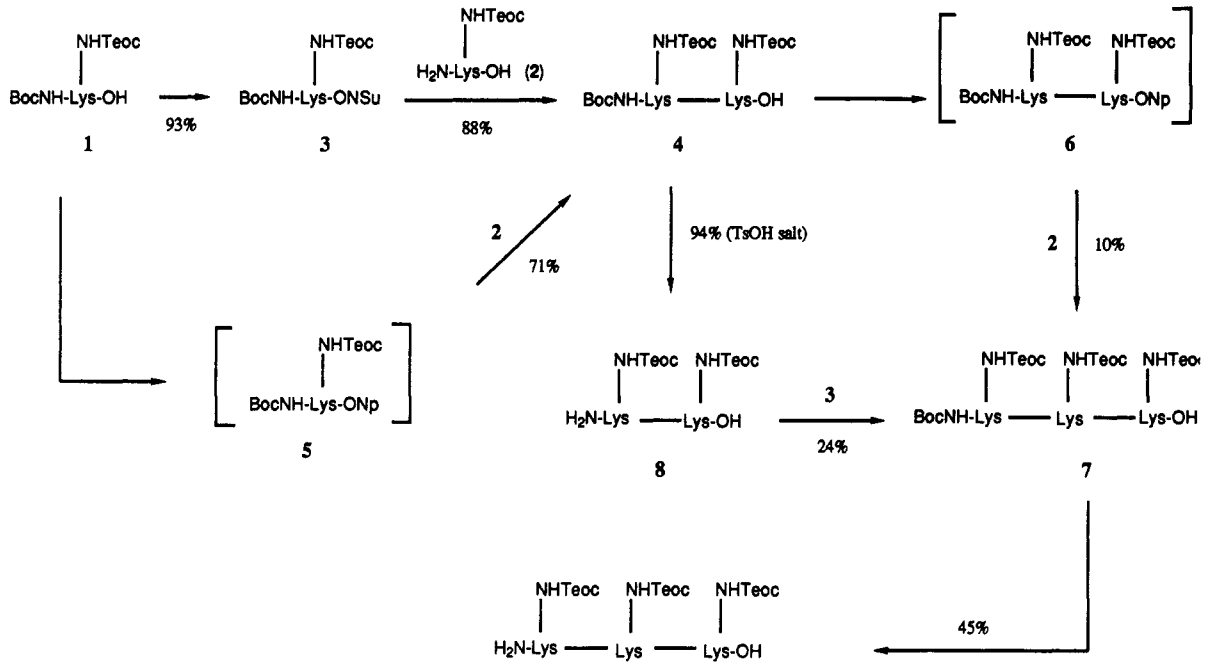
(1) Albert, A. *Selective Toxicity*, 5th ed.; Chapman and Hall: London, 1973.
(2) Donaruma, I. G.; Vogl, O. *Polymeric Drugs*; Acdemic Press: New York, 1978.
(3) Gros, L.; Ringsdorf, H.; Schupp, H. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 305.
(4) van Oss, C. J.; Gillman, C. F.; Newmen, H. J. P. *Phagocytic Engulfment and Cell Adhesiveness*; Marcel Dekker: New York, 1975.
(5) Arnold, L. J. *Meth. Enzymol.* **1985**, *112*, 270.
(6) Przybylski, M.; Fell, E.; Ringsdorf, H.; Zaharko, D. S. *Makromol. Chem.* **1978**, *179*, 1719.
(7) (a) Ryser, H. J.-P.; Shen, W.-C. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3867. (b) Shen, W.-C.; Ryser, H. J.-P. *Mol. Pharmacol.* **1979**, *16*, 614. (c) Shen, W.-C.; Ryser, H. J.-P. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 7589.
(8) Chu, B. C. F.; Howell, S. B. *Biochem. Pharmacol.* **1981**, *30*, 2545. (b) Chu, B. C. F.; Howell, S. B. *Biochem. Pharmacol.* **1982**, *31*, 3513.
(9) (a) Whiteley, J. M.; Nimec, Z.; Galivan, J. *Mol. Pharmacol.* **1981**, *19*, 505. (b) Galivan, J.; Balinska, M.; Whiteley, J. M. *Arch. Biochem. Biophys.* **1982**, *216*, 544.
(10) Rosowsky, A.; Forsch, R. A.; Galivan, J.; Susten, S. S.; Freisheim, J. H. *Mol. Pharmacol.* **1985**, *27*, 141.
(11) Forsch, R.; Rosowsky, A. *J. Org. Chem.* **1984**, *49*, 1305.
(12) Rosowsky, A.; Forsch, R. A.; Freisheim, J. H.; Galivan, J.; Wick, M. *J. Med. Chem.* **1984**, *27*, 888.
(13) Our attention was first drawn to this possible route to drug–homopolypeptide conjugates of specified structure by a paper in which a block polymer strategy was used to prepare polymers such as $(Tyr\text{-}LysLysLys)_n$, see: Fuller, W. D.; Verlander, M. S.; Goodman, M. *Biopolymers* **1976**, *15*, 1869.
(14) A trilysine derivative with the ϵ-amino group on the N-terminal joined to the γ-carboxyl of α-benzyl MTX, a *tert*-butyloxycarbonyl (Boc) group on the terminal α-amino group, and the other ϵ-amino groups blocked with benzyloxycarbonyl (Cbz) groups was reported by us earlier[11] and could have been used, in principle, in a block polymer synthesis after cleavage of the Boc group. However, the length of this peptide building block was judged to be insufficient, and we chose to focus instead on tetra-L-lysines. We also considered the Teoc group to be more attractive for ϵ-amino group protection because of the relative ease with which it could be removed using fluoride ion.[12]
(15) Rosowsky, A.; Wright, J. E. *J. Org. Chem.* **1983**, *48*, 1539.

0022-3263/89/1954-5551$01.50/0

Scheme I



Scheme II

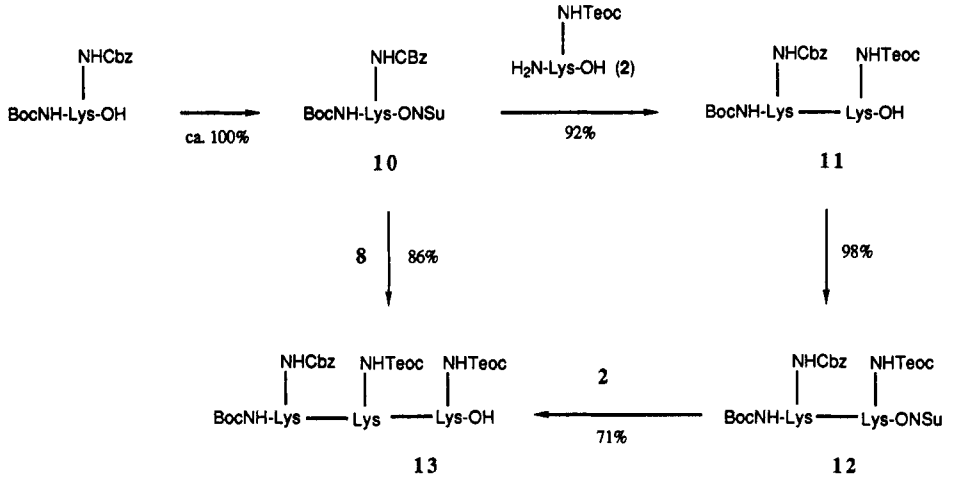


groups. Scheme I outlines a possible retrosynthetic strategy, using MTX as the ligand. One blocking group (R^1) is used for the α-amino group of the N-terminal lysine, a second (R^2) for the ϵ-amino group of the N-terminal lysine, and a third (R^3) for all remaining ϵ-amino groups. Selective removal of R^2, with retention of R^1 and R^3, allows coupling of the drug to the N-terminal lysine. After removal of R^1, this time with preservation of R^3, the product is subjected to block polymerization with the aid of an appropriate peptide bond forming reagent, e.g. diphenyl phosphorazidate, and R^3 protecting groups are cleaved under conditions known to leave the drug–polymer bond intact.

We considered first the possibility of using a *tert*-butyloxycarbonyl (Boc) group at R^1, a benzyloxycarbonyl (Cbz) group at R^2, and a [2-(trimethylsilyl)ethoxy]carbonyl (Teoc) group at R^3. It was reasoned that R^2 could be removed by hydrogenolysis without loss of R^1 or R^3 and that, after attachment of the ligand to the ϵ-amino group of the N-terminal lysine, R^1 could be removed under mild acid conditions without loss of R^3. The basis for this expectation was our earlier finding that N^{α}-Boc-N^{ϵ}-Teoc-L-lysine (**1**) could be converted to N^{ϵ}-Teoc-L-lysine (**2**) in good yield by mild treatment with *p*-toluenesulfonic acid.[15] Accordingly we converted **1** to di- and tripeptide model compounds containing the desired triad of blocking groups (R^1 = Boc, R^2 = Cbz, R^3 = Teoc) by the reactions shown in Schemes II and III. Activation of the COOH group in **1** by reaction with *N*-hydroxysuccinimide (HOSu) and *N,N'*-dicyclohexylcarbodiimide (DCC)[16] afforded the ester **3** (93%), which on reaction with **2** in the presence of *N*-ethyl-*N,N*-diisopropylamine (EDPA) was converted to the Boc/Teoc/Teoc dipeptide **4** (88%). Alternatively, **4** could be made from **1** by treatment with bis(*p*-nitrophenyl) carbonate[17] followed, without isolation of the activated ester **5**, by reaction with **2** and EDPA. The overall yield by the latter route was 71%. Selective removal of the Boc group in **4** was accomplished with *p*-toluenesulfonic acid in ether,[15,18] giving **8**·TsOH in 94% yield. The corresponding 2,4,6-trimethylbenzenesulfonate salt was also prepared, albeit in lower yield, by using 2,4,6-trimethylbenzenesulfonic acid in ethyl acetate. It thus appeared that selective α-Boc cleavage was possible in the peresence of not only one but two ϵ-Teoc groups. Reaction of the OSu ester **3** with **8** in DMF solution at room temperature afforded the tripeptide **7**, but the yield was only 24%. In an effort to improve the yield of **7**, dipeptide **4** was activated with bis(*p*-nitrophenyl) carbonate and EDPA, and

(16) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1964**, *86*, 1839.
(17) Wieland, T.; Heinke, B.; Vogeler, K.; Morimoto, H. *Justus Liebigs Ann. Chem.* **1962**, *655*, 189.
(18) Goodacre, J.; Ponsford, R. J.; Stirling, I. *Tetrahedron Lett.* **1975**, 3609.

Scheme III



the resultant ester, **6**, was allowed to react in situ with **2**. Unfortunately the yield of **7** by this alternative route was poor (10%); moreover, subsequent removal of the Boc group from **7** with *p*-toluenesulfonic acid afforded a yield (45%) substantially lower than that of **3** from **1** or **8** from **4**. TLC analysis revealed the presence of a number of byproducts in the acidolysis, which we assumed were due to loss of one or more Teoc groups. These results demonstrated that selective Boc cleavage is possible in the presence of Teoc groups, but only when the Boc/Teoc ratio is 1:1 or 1:2. The Boc/Teoc combination therefore should not be viewed as a general strategy for orthogonal protection in peptide synthesis.

Though we were aware of the limitations of the Boc/Teoc combination, we nonetheless felt that it would be of interest to prepare a trilysine with one Boc group, two Teoc groups, and one Cbz group, as in compound **13** (Scheme III). This model compound was selected with the idea that the Cbz group could be removed selectively by hydrogenolysis, allowing a single molecule of MTX to be attached to the ϵ-amino group of the N-terminal residue as outlined in Scheme I. The activated ester **10** was prepared in essentially quantitative yield from N^{α}-(*tert*-butyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysine, HOSu, and DCC and was condensed with **2** in the presence of EDPA to obtain the Boc/Cbz/Teoc dipeptide **11** (92%) or with **8** to obtain the tripeptide **13** (86%). Alternatively, **13** could be prepared from **11** by conversion to the activated ester **12** (98%) followed by reaction with **2**; the latter reaction proceeded in 71% yield and was therefore less efficient than that of **10** with **8**.

These results demonstrated the feasibility of regiospecific protection with Boc, Teoc, and Cbz groups, provided that no more than two Teoc groups were present. Since our intent was to eventually prepare tetra- or pentalysines as drug carriers for block polymerization, we abandoned the Boc group and adopted the 9-fluorenylmethyloxycarbonyl (Fmoc) group[19] for protection of the α-amino group in the N-terminal lysine. We reasoned that it should be possible to remove the N^{ϵ}-Cbz group from the N-terminal lysine residue by catalytic hydrogenation without loss of the N^{α}-Fmoc group[19] and that subsequent acylation of the single unprotected ϵ-amino group and cleavage of the Fmoc group with liquid ammonia[19] should proceed without any loss of N^{ϵ}-Teoc groups.[19a] In peptide synthesis not involving solid-phase methods, liquid ammonia appears to be a convenient alternative to piperidine[20] for Fmoc cleavage.

Removal of Fmoc groups in the presence of Teoc groups was initially demonstrated in model compounds lacking a Cbz group (Scheme IV). N^{α}-(9-Fluorenylmethyloxycarbonyl)-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine (**14**) was prepared in 81% yield from **2** and 9-fluorenylmethyl chloroformate[19] and was converted to the *p*-nitrophenyl ester **15** (89%) on reaction with *p*-nitrophenyl chloroformate in the presence of a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine (DMAP). Alternatively, N^{α}-(9-fluorenylmethyloxycarbonyl)-L-lysine was converted to **15** in 60% yield via a one-pot synthesis involving heating with 2-(trimethylsilyl)ethyl *p*-nitrophenyl carbonate[15] and EDPA in DMF solution at 55–60 °C followed by treatment in situ with DCC at room temperature.[21] Reaction of **15** with **2** afforded the fully blocked dilysine derivative **16** (60%), which on treatment with liquid ammonia in a Teflon-lined stainless steel autoclave at room temperature for 2.5 days was converted to **8** (84%). Similarly, condensation of **15** and **8** led to the Fmoc/Teoc tripeptide **17** (71%), which on Fmoc cleavage with ammonia yielded **9** (97%) without any loss of Teoc groups.

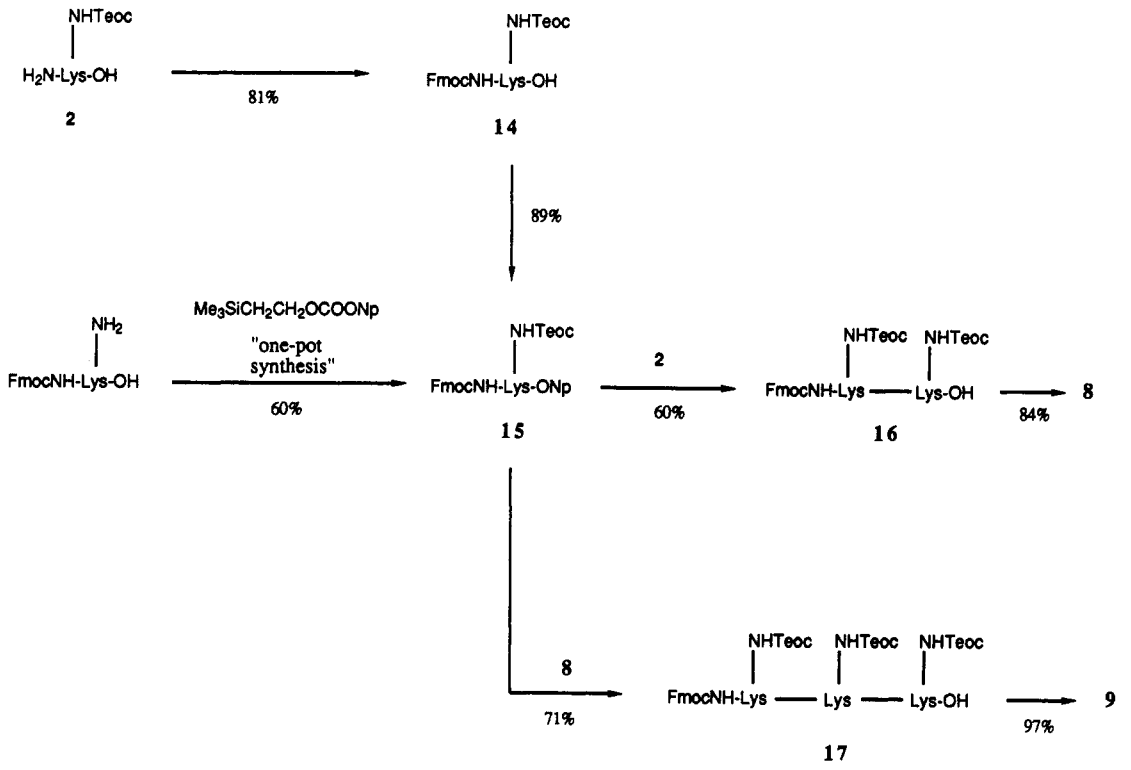
Since it was clear from these results that Fmoc and Teoc groups were more compatible than Boc and Teoc groups, we next embarked on the synthesis of tri- and tetralysines with amino groups protected by Fmoc, Cbz, and Teoc groups (Scheme V). N^{ϵ}-(Benzyloxycarbonyl)-L-lysine was acylated with 9-fluorenylmethyl chloroformate, and the resultant product, **18** (64%), was converted to its *p*-nitrophenyl ester (**19**) in 95% yield by reaction with *p*-nitrophenyl chloroformate, EDPA, and a catalytic amount of DMAP. Condensation of **19** with **8** in the presence of EDPA and DMAP, or with **9** in the presence of EDPA alone, led to the fully protected Fmoc/Cbz/Teoc derivatives **20** (87%) and **22** (57%), respectively. Catalytic hydrogenolysis of the Cbz group then gave the partially deprotected tri- and tetralysines **21** (65%) and **23** (81%) with the Fmoc and Teoc groups still in place. While we had some concern, in undertaking these reactions, that it might not be possible to remove the Cbz group selectively in **21**

(19) Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404. (a) An alternative, and probably more selective, strategy using Cbz/Fmoc protection would have been to synthesize analogues of **20** and **21** with Fmoc on the ϵ-amino and Cbz on the α-amino group of the N-terminal lysine. This would have allowed deblocking of the ϵ-amino group with liquid ammonia or piperidine. We are grateful to one of the reviewers for correctly pointing this out.

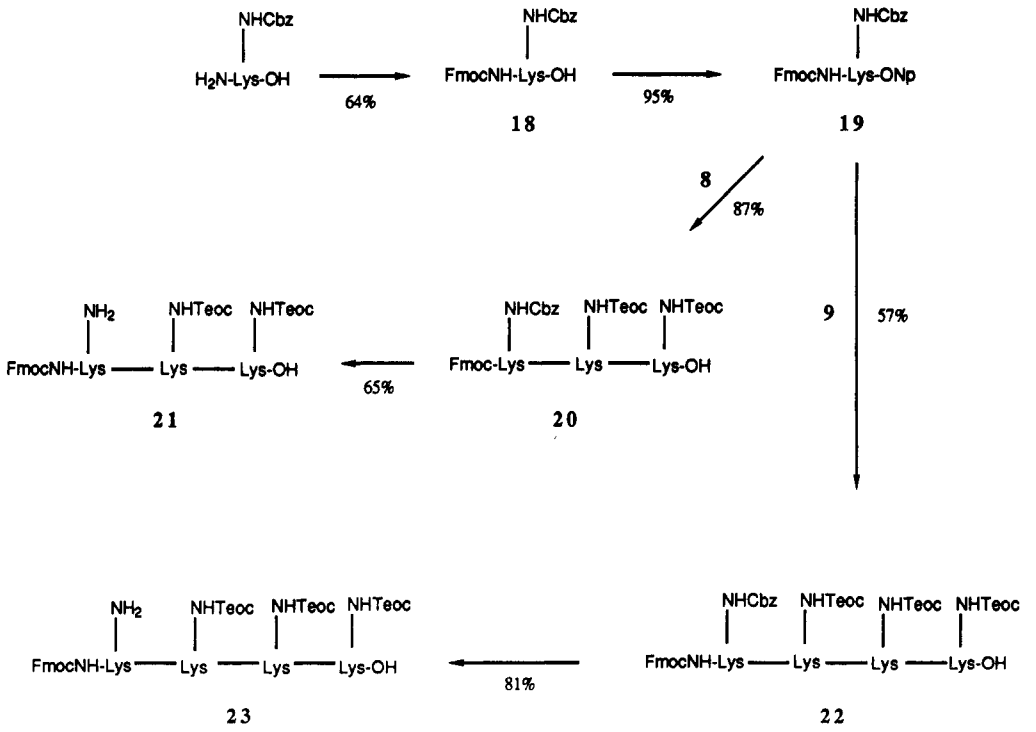
(20) Chang, C.-D.; Waki, M.; Ahmad, M.; Meienhofer, J.; Lundell, E. O.; Haug, J. D. *Int. J. Peptide Protein Res.* **1980**, *15*, 59.

(21) The principle behind this convenient, though rarely used, procedure is that the *p*-nitrophenol released on N-acylation reacts with the carboxyl group once the DCC is added; see, for example: Wolman, Y.; Ladkany, D.; Frankel, M. *J. Chem. Soc. C* **1967**, 689.

Scheme IV



Scheme V



and **23**, this proved not to be a problem even though there is literature precedent for the hydrogenolysis of Fmoc groups.[22]

In summary, these model experiments demonstrate the feasibility of preparing di-, tri-, and tetra-L-lysine derivatives with Teoc groups on all the ϵ-amino groups as well as on all but the N-terminal residue. The Fmoc group appears to be superior to the Boc group as a means of protection of the N-terminal α-amino group in oligo(L-lysines) containing more than two lysine residues. Fmoc cleavage can be achieved readily in these oligo(L-lysines) under nonacidic conditions which are compatible with Teoc groups, whereas Boc cleavage requires acid treatment and is likely to be accompanied by partial loss of Teoc. The Fmoc group is also advantageous because it allows chromatographic separations to be monitored on the basis of UV absorbance. The Cbz group is compatible with both Fmoc and Teoc groups and thus is well-suited to synthetic

(22) (a) Atherton, E.; Bury, C.; Sheppard, R. C.; Williams, B. J. *Tetrahedron Lett.* **1979**, 304. (b) Martinez, J.; Tolle, N. C.; Bodanszky, M. *J. Org. Chem.* **1979**, *44*, 3596.

schemes involving the three different blocking groups and an orthogonal deprotection strategy.[23] Further studies are needed to assess the ability of lysyl oligopeptides such **21** and **23** to serve as building blocks for the synthesis of structurally defined drug conjugates of poly(L-lysine).

Experimental Section

Infrared spectra were obtained on a Perkin-Elmer Model 781 double-beam recording spectrophotometer, and NMR spectra were obtained on a Varian T60A instrument with Me_4Si as the internal standard, except in the case of compounds already containing a singlet for one or more Me_3Si groups, where this singlet was used as the internal reference and was set at δ 0.00. Spectral data consistent with the assigned structures were obtained for all compounds. TLC separations were carried out on Baker Si250F plates (system A, 1:1:0.01 hexanes–EtOAc–AcOH), Whatman MK6F plates (system B, 9:1 $CHCl_3$–MeOH; system C, 1:4 EtOAc–hexanes; system D, 79:20:1 EtOAc–hexanes–AcOH; system E, 74:25:1 EtOAc–hexanes–AcOH; system F, 12:7:1 EtOAc–EtOH–28% NH_4OH; system G, 13:6:1 $CHCl_3$–EtOH–28% NH_4OH), or MKC18F plates (system H, 1:1 Me_2CO–H_2O; system I, 3:2 Me_2CO–H_2O; system J, 2:1 Me_2CO–H_2O). Letters in parentheses after the R_f values in the experimental section refer to the developing systems listed above. Spots were visualized under 254-nm illumination in a viewing chamber except for compounds lacking a UV-absorbing group. In those instances a convenient method of visualization was to leave the plate in a closed TLC tank containing trifluoroacetic acid vapor and then remove the plate from the tank and spray it with ninhydrin. Column chromatography was carried out on Baker 3405 (60–200 mesh) and Baker 7024-1 "Flash" silica gel (40 ± 15 μm diameter particle size) (J. T. Baker, Phillipsburg, NJ), or on Sephadex LH20 (Pharmacia, Piscataway, NJ). N^{α}-(*tert*-Butyloxycarbonyl)-L-lysine, N^{ϵ}-(benzyloxycarbonyl)-L-lysine, N^{α}-(*tert*-butyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysine, and N^{α}-(9-fluorenylmethyloxycarbonyl)-L-lysine were purchased from Chemical Dynamics, South Plainfield, NJ, and Bachem, Torrance, CA. N^{α}-(*tert*-Butyloxycarbonyl)-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine (**1**) and N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine (**2**) were synthesized as reported earlier.[15] Other chemicals were from Aldrich, Milwaukee, WI. Solvents used in coupling reactions were dried over Linde 4A molecular sieves (Fisher, Boston, MA). Melting points were measured in Pyrex capillary tubes in a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, MA) or on a Fisher-Johns hot-stage apparatus (Fisher, Boston, MA). Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, or MultiChem Laboratories, Lowell, MA, and were within ±0.4% of theoretical values unless otherwise indicated.

N^{α}-(*tert*-Butyloxycarbonyl)-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine *N*-Hydroxysuccinimide Ester (3). HOSu (1.84 g, 16 mmol) and DCC (3.35 g, 16.2 mmol) were added to a solution of N^{α}-(*tert*-butyloxycarbonyl)-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine (5.86 g, 15 mmol) in EtOAc (80 mL), and the mixture was stirred at room temperature for 24 h, diluted to 200 mL with Et_2O, and filtered. Evaporation of the filtrate and drying of the residue overnight in vacuo at 25–30 °C gave a glassy solid (6.82 g, 93%): mp 40–43 °C; TLC R_f 0.24 (J). Anal. Calcd for $C_{21}H_{37}N_3SiO_8$: C, 51.72; H, 7.65; N, 8.62; Si, 5.76. Found: C, 51.73; H, 7.82; N, 8.88; Si, 5.50.

N^{α}-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine (4). Method A. Hydroxysuccinimide Ester Coupling. To a suspension of **2** (2.58 g, 8.87 mmol) in CH_2Cl_2 (100 mL) were added EDPA (1.55 mL, 1.15 g, 8.87 mmol) and **3** (3.68 g, 10.6 mmol), and the mixture was stirred at 25–30 °C for 3 days and evaporated to an oil, which was chromatographed on a Sephadex LH20 column (5 cm i.d. × 40 cm) with 95% EtOH as the eluent. Fractions were monitored for product by TLC and appropriately pooled and evaporated to dryness. The residue was redissolved in 30:1 Et_2O–hexanes, and, after filtration to restore clarity, the filtrate was extracted with 0.5 N HCl (100 mL) to remove any remaining EDPA. The organic phase was dried ($MgSO_4$) and evaporated. The residue was kept in vacuo at 25–30 °C for 18 h to obtain a glassy solid (5.43 g, 88%): mp 48–50 °C; TLC R_f 0.19 (D), R_f 0.46 (F), R_f 0.50 (I).

Method B. *p*-Nitrophenyl Ester Coupling (One-Pot Synthesis). A solution of **1** (1.16 g, 2.97 mmol) and bis(*p*-nitrophenyl) carbonate (1.08 g, 3.55 mmol) in a mixture of EtOAc (30 mL) and pyridine (1 mL) was heated under reflux for 4 h, at which time TLC (system D) showed formation of *p*-nitrophenol (R_f 0.81) and **5** (R_f 0.88, not isolated), with disappearance of all bis(*p*-nitrophenyl) carbonate (R_f 0.94) and **1** (R_f 0.45). To the solution were then added **2** (0.83 g, 2.87 mmol) and EDPA (0.5 mL), and refluxing was continued overnight. Solvents were evaporated under reduced pressure at 50 °C, and the residue was partitioned between EtOAc (100 mL) and 0.3 N HCl (100 mL). The EtOAc layer was extracted with 2 M K_2CO_3 (6 × 75 mL), dried (Na_2SO_4), concentrated to a volume of 15 mL, and applied onto a silica gel column (5 cm × 50 cm). Elution was performed with EtOAc (1.5 L) followed by 2% AcOH in EtOAc (1.5 L). Appropriate fractions of the latter eluent were pooled, washed with saturated $NaHCO_3$, dried (Na_2SO_4), and evaporated to a foam, which was dried at room temperature in vacuo to remove final traces of solvent: yield 1.34 g (71%); TLC R_f 0.19 (D).

IR and NMR spectra of **4** prepared by methods A and B were essentially the same. Microanalytical data were obtained on a sample prepared by method A. Anal. Calcd for $C_{29}H_{58}N_4Si_2O_9 \cdot 0.25Et_2O \cdot 0.75H_2O$: C, 51.84; H, 8.99; N, 8.06; Si, 8.08. Found: C, 51.94; H, 9.06; N, 8.05; Si, 8.03.

N^{α}-[N^{α}-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine (7). Method A. *N*-Hydroxysuccinimide Ester Coupling. EDPA (0.5 mL) was added to a solution of 8·TsOH (see below) (0.746 g, 1.07 mmol) and **3** (0.546 g, 1.12 mmol) in DMF (25 mL), and the solution was stirred at room temperature for 2 days and evaporated under reduced pressure. The residue was chromatographed on a silica gel column (20 cm i.d. × 30 cm) with 3:1 $CHCl_3$–MeOH as the eluent. Product fractions were pooled and evaporated, and residual solvent was removed by entrainment with Et_2O (3 × 100 mL) followed by drying in vacuo for 24 h to obtain a foam (0.24 g, 24%): TLC R_f 0.12 (I).

Method B. *p*-Nitrophenyl Ester Coupling (One-Pot Synthesis). A solution of **4** (2.28 g, 5.83 mmol), bis(*p*-nitrophenyl) carbonate (1.80 g, 5.92 mmol), and EDPA (0.5 mL) in DMF (4 mL) was heated at 65–70 °C for 4 h under reduced pressure with exclusion of moisture and was then cooled to room temperature. To the solution, whose TLC showed the presence of the active ester **6** (not isolated), was added **2** (1.70 g, 5.86 mmol) followed by a second portion of EDPA (0.5 mL). Heating was resumed under reduced pressure at 60–65 °C for 2 days. After addition of a second portion of bis(*p*-nitrophenyl) carbonate (1.80 g, 5.92 mmol) to form the active ester **6** (not isolated), the mixture was heated to reflux for 4 h. A second portion of **2** (1.70 g, 5.86 mmol) was then added, and refluxing was allowed to continue for 3 days. After evaporation under reduced pressure at 60 °C, the residue was taken up in EtOAc (75 mL), and the isolation was washed successively with 0.08 N HCl (2 × 100 mL), saturated $NaHCO_3$ (100 mL), and distilled H_2O (100 mL). The organic layer was dried (Na_2SO_4) and concentrated to 15 mL, and the solution was applied onto a silica gel column (400 g, 5 cm i.d. × 40 cm) with 50:10:1 $CHCl_3$–EtOH–28% NH_4OH as the eluent. Appropriate fractions [TLC: R_f 0.58 (F)] were pooled and evaporated, and residual solvents were removed by entrainment with EtOAc (3 × 20 mL) followed by drying in vacuo for 9 days; yield 0.539 g (10%).

IR and NMR spectra of **7** prepared by methods A and B were essentially identical. Microanalytical data were obtained on a sample prepared by method B. Anal. Calcd for $C_{41}H_{82}N_6Si_3O_{12}$: C, 52.65; H, 8.84; N, 8.98; Si, 9.01. Found: C, 52.40; H, 8.80; N, 8.48; Si, 9.03.

N^{α}-[N^{ϵ}-[[2-(Trimethylsilyl)ethoxy]carbonyl]-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine (8). A. Preparation of the *p*-Toluenesulfonate Salt (8·TsOH). *p*-TsOH·H_2O (0.272 g, 1.43 mmol) was added to a solution of **4** (0.988 g, 1.42 mmol) in Et_2O (10 mL) at room temperature, and as soon as all the solid was dissolved the solution was evaporated to dryness under reduced pressure at 20–27 °C. The residue was kept at 27–30 °C for 45 min, during which there was vigorous

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effervescence. Storage in vacuo for 2.5 days at 25–30 °C gave an oil (1.07 g, 94%): TLC R_f 0.56 (J). Anal. Calcd for $C_{24}H_{50}N_4Si_2O_7 \cdot CH_3C_6H_4SO_3H \cdot 0.25H_2O$: C, 50.28; H, 8.10; N, 7.56; Si, 7.58; S, 4.33. Found: C, 50.59; H, 8.31; N, 7.38; Si, 7.14; S, 4.33.

B. Preparation of the 2,4,6-Trimethylbenzenesulfonate Salt (8·TmbsOH). 2,4,6-Trimethylbenzenesulfonic acid monohydrate (1.1 g, 4.65 mmol) was added to a solution of **4** (3.15 g, 4.21 mmol) in EtOAc (50 mL), and, after overnight stirring at room temperature, the solvent was evaporated under reduced pressure at 70 °C. Trituration with 28% NH_4OH (10 mL), followed by decantation and removal of excess ammonia by entrainment with EtOH (25 mL) under reduced pressure, gave a crude product from which some unreacted **4** was removed by trituration with Et_2O (25 mL). Drying of the residue from Et_2O trituration for 3 h in vacuo left a solid (1.89 g, 56%): mp 168–170 °C. Anal. Calcd for $C_{24}H_{50}N_4Si_2O_7 \cdot C_9H_{15}SO_3 \cdot 0.5H_2O$: C, 50.23; H, 8.43; N, 8.87. Found: C, 50.57; H, 8.17; N, 8.55.

C. Preparation of the Free Base. A solution of **16** (see below) (1.01 g, 1.29 mmol) in liquid NH_3 (50 mL) at −40 °C was sealed in a 250-mL Teflon-lined stainless-steel autoclave, allowed to come to room temperature, and left to stand for 2.5 days. The pressure was released slowly, the NH_3 was allowed to evaporate, and the residue was triturated with petroleum ether (3 × 100 mL) and filtered. The solid was then taken up in Et_2O, a small amount of insoluble material was removed by filtration, the Et_2O filtrate was added to petroleum ether (200 mL), and the solution was concentrated to a volume of 120 mL. The precipitate was collected, washed with petroleum ether (25 mL), and dried in vacuo overnight at room temperature to obtain a colorless solid (0.602 g, 84%): mp 192–195 °C; TLC R_f 0.14 (G). Anal. Calcd for $C_{24}H_{50}N_4Si_2O_7$: C, 51.21; H, 8.95; N, 9.95. Found: C, 51.14; H, 8.99; N, 9.94.

N^{α}-[N^{ϵ}-[[2-(Trimethylsilyl)ethoxy]carbonyl]-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine (9). Method A. 2,4,6-Trimethylbenzenesulfonic acid dihydrate (0.206 g, 8.45 mmol) was added to a solution of **7** (0.784 g, 0.838 mmol) in Et_2O (20 mL), and the mixture was stirred until all the solid dissolved. The clear solution was evaporated to dryness under reduced pressure, and the residue was heated for 1 h at 70–75 °C. The resulting solid was triturated with Et_2O (2 × 30 mL) and kept for 4.5 h in vacuo at 35–40 °C to obtain a product (0.463 g) which was impure according to TLC (system G). A portion (250 mg) of the product was chromatographed on a silica gel column (2.8 cm i.d. × 36 ccm), which was packed with EtOAc and eluted with 60:35:5 EtOAc–EtOH–28% NH_4OH. Fractions of 8 mL were monitored by TLC, and those found to be homogeneous were pooled and evaporated. Fraction 1 (tubes 17–27) yielded unchanged **7** (40 mg, 5% recovery); fraction 2 (tubes 37–65) yielded **9**, as the free base (169 mg); fraction 3 (tubes 69–240) yielded another product (41 mg) whose NMR spectrum was consistent with the presence of only two Teoc groups. The yield of **9** calculated on the basis of the purification of the 250-mg portion of crude product (out of a total of 463 mg) was 45%.

Method B. Deprotection of **17** (see below) (0.66 g, 0.83 mmol) in liquid NH_3 (25 mL) was performed as described for **16**: yield 0.53 g (97%); mp 153–155 °C; TLC R_f 0.36 (G). The product obtained by this procedure was the same as the one prepared by method A. Anal. Calcd for $C_{36}H_{74}N_6Si_3O_{10} \cdot 0.5H_2O$: C, 51.24; H, 8.94; N, 9.95; Si, 9.97. Found: C, 51.20; H, 8.93; N, 9.60; Si, 10.23.

The sulfonate salts as well as free base forms of **8** and **9** were hygroscopic. Their melting points varied from batch to batch and on damp days were substantially depressed.

N^{α}-(*tert*-Butyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysine *N*-Hydroxysuccinimide Ester (10). To a solution of N^{α}-(*tert*-butyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysine (1.15 g, 3 mmol) in EtOAc (5 mL) were added HOSu (0.358 g, 3.1 mmol) and DCC (0.674 g, 3.27 mmol), and the solution was stirred at 25 °C for 3 h. The precipitated *N,N'*-dicyclohexylurea (DCU) was removed by filtration, and the filtrate was concentrated to 1 mL under reduced pressure. Et_2O (10 mL) was added, the mixture was stirred overnight, an additional amount of DCU was removed by filtration, and the filtrate was evaporated. Removal of residual EtOAc by entrainment with *n*-heptane (2 × 10 mL) under reduced pressure, followed by overnight drying in vacuo at 75–80 °C afforded a glassy solid (1.57 g, 100% yield) which was pure enough to use in the next step. An analytical sample was prepared by redissolving the product in EtOAc, extracting the solution with an equal volume of 0.1 N $NaHCO_3$ (100 mL), rinsing twice with an equal volume of H_2O (2 × 100 mL), evaporating the organic layer to dryness, and keeping the residue in vacuo at 35–40 °C overnight. Trituration with 10:1 Et_2O–hexanes yielded a white solid, which was dried in vacuo for 4 h at 45–50 °C: mp 110–111 °C; TLC R_f 0.38 (A). Anal. Calcd for $C_{23}H_{31}N_3O_8$: C, 57.84; H, 6.56; N, 8.80. Found: C, 57.61; H, 6.39; N, 8.60.

N^{α}-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine (11). To a suspension of **2** (0.29 g, 1 mmol) in dry DMF (25 mL) were added EDPA (0.2 mL) and **10** (0.523 g, 1.1 mmol), and after being stirred at 25 °C for 5 days the mixture was evaporated to dryness. The residue was taken up in MeOH (8 mL), and the solution was applied onto a Sephadex LH20 column (2.8 cm i.d. × 40 cm), which was eluted with MeOH. Fractions of 7.5 mL were monitored by TLC (H) for the presence of N^{α}-(*tert*-butyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysine (R_f 0.66), **2** (R_f 0.37), and **11** (R_f 0.24). Fractions containing only **11** were pooled and evaporated, residual MeOH was removed by entrainment with Et_2O (3 × 5 mL), and the resulting foam was dried in vacuo for 2 h: yield 0.6 g (92%); mp 58–60 °C. Anal. Calcd for $C_{31}H_{52}N_4SiO_9$: C, 57.03; H, 8.03; N, 8.58; Si, 4.30. Found: C, 56.74; H, 8.21; N, 8.56; Si, 4.12.

N^{α}-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine *N*-Hydroxysuccinimide Ester (12). To a solution of **11** (1.05 g, 1.61 mmol) in EtOAc (10 mL) were added HOSu (1.85 g, 1.61 mmol) and DCC (0.333 g, 1.61 mmol), the mixture was stirred at 25–30 °C for 3 days, and Et_2O (20 mL) was added. The precipitated DCU was removed by filtration, and the filtrate was evaporated to an oil. Residual EtOAc was removed by entrainment with Et_2O (2 × 25 mL) under reduced pressure, and the residue was dried in vacuo at 25–30 °C for 5 h: yield 1.19 g (98%); mp 49–51 °C. Anal. Calcd for $C_{35}H_{55}N_5SiO_{11}$: C, 56.06; H, 7.39; N, 9.34; Si, 3.74. Found: C, 56.28; H, 7.54; N, 9.23; Si, 3.85.

N^{α}-[N^{α}-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine (13). Method A. To a suspension of **2** (0.29 g, 1 mmol) in dry DMF (15 mL) were added **12** (0.29 g, 1 mmol) and EDPA (0.2 mL), and the mixture was kept in an ultrasonic bath, with occasional trituration, for 1.5 h to obtain a fine dispersion. The mixture was stirred at 25–30 °C for 8 days and evaporated to dryness under reduced pressure at 60 °C. The residue was taken up in MeOH, and the solution was chromatographed on Sephadex LH20 as described for compound **4**. TLC-homogeneous fractions, R_f 0.27 (J), were pooled and evaporated to obtain a colorless solidified foam (0.653 g, 71%).

Method B. EDPA (0.27 mL) was added to a solution of **8**·TsOH (0.584 g, 0.788 mmol) and **10** (0.741 g, 1.58 mmol) in dry DMF (10 mL), and mixture was stirred at room temperature for 20 h and evaporated to dryness under reduced pressure at 55–60 °C in the presence of 0.4 M Na_2CO_3 (1 mL). The residue was chromatographed on a Sephadex LH20 column (5 cm i.d. × 35 cm) with 95% EtOH as the eluent. Fractions containing only **13** were pooled and evaporated, and the product was dried in vacuo at room temperature overnight: yield 0.63 g (86%); mp 64–67 °C; TLC R_f 0.27 (J).

The same product was obtained by methods A and B. Microanalytical data were obtained on material prepared by method B. Anal. Calcd for $C_{43}H_{76}N_6Si_2O_{12}$: C, 55.82; H, 8.28; N, 9.08; Si, 6.07. Found: C, 55.80; H, 8.37; N, 8.87; Si, 5.91.

N^{α}-(9-Fluorenylmethyloxycarbonyl)-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine (14). To a stirred solution of **2** (4.35 g, 15 mmol) in a mixture of dioxan (15 mL) and 1 M K_2CO_3 (30 mL) at 0–4 °C was added over 30 min a solution of 9-fluorenylmethyl chloroformate (3.89 g, 15 mmol) in dioxan (30 mL). The mixture was allowed to come room temperature, stirred overnight, diluted with H_2O (200 mL), and extracted with Et_2O (2 × 100 mL). The aqueous layer was acidified to pH 5.5 with 0.5 N HCl and extracted with $CHCl_3$ (3 × 100 mL). The $CHCl_3$ layer was dried (Na_2SO_4), evaporated, and dried in vacuo for

several days at room temperature to obtain a solidified foam (6.2 g, 81%): mp 102–104 °C; TLC R_f 0.38 (E), R_f 0.27 (G). Anal. Calcd for $C_{27}H_{36}N_2SiO_6$: C, 63.26; H, 7.08; N, 5.46; Si, 5.48. Found: C, 63.04; H, 7.16; N, 5.30; Si, 5.80.

p-Nitrophenyl N^{α}-(9-Fluorenylmethyloxycarbonyl)-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysinate (15). Method A. EDPA (0.35 mL) was added to a solution of *p*-nitrophenyl chloroformate (0.439 g, 2.11 mmol) and **14** (1.03 g, 2.00 mmol) in CH_2Cl_2 (10 mL) at 0 °C. After 10 min of stirring, DMAP (30 mg) was added, and stirring was continued for another 30 min. The reaction mixture was diluted to 50 mL with CH_2Cl_2, and the solution was extracted with saturated $NaHCO_3$ (6 × 100 mL) followed by 0.15 N HCl (2 × 100 mL) and saturated NaCl (100 mL). The CH_2Cl_2 layer was dried (Na_2SO_4) and passed through a short column (3 cm) of silica gel. The silica gel bed was washed with CH_2Cl_2, the combined filtrate and washed solution were evaporated, and the residue was recrystallized from Et_2O to obtain a yellow solid (1.12 g, 89%): mp 106–108 °C; TLC R_f 0.26 (C).

Method B. (One-Pot Synthesis). A solution of N^{α}-(9-fluorenylmethyloxycarbonyl)-L-lysine (7.37 g, 20 mmol), 2-(trimethylsilyl)ethyl *p*-nitrophenyl carbonate (6.32 g, 2.3 mmol), and EDPA (3.5 mL) in DMF (30 mL) was heated at 50–55 °C for 2.5 h. The reaction mixture was cooled to room temperature, and DCC (4.54 g, 22 mmol) was added. A precipitate formed soon thereafter. The mixture was stirred for 30 min and evaporated to dryness under reduced pressure, and the residue was treated with CH_2Cl_2 (50 mL). The insoluble DCU was filtered off, and the filtrate was evaporated at 70–75 °C under reduced pressure. The residue was taken up in Et_2O (50 mL), and the Et_2O solution was extracted with 1 M K_2CO_3 (3 × 30 mL), rinsed with distilled H_2O (3 × 50 mL), dried (Na_2SO_4), and diluted to 300 mL with hexanes. The copious yellow precipitate was collected, washed with hexanes (100 mL), and dried in vacuo overnight; yield 7.65 g (60%).

The same product was obtained by methods A and B. Microanalytical data were obtained on material prepared by method A. Anal. Calcd for $C_{33}H_{39}N_3SiO_8$: C, 62.54; H, 6.20; N, 6.63; Si, 4.43. Found: C, 62.75; H, 6.31; N, 6.38; Si, 4.58.

N^{α}-[N^{α}-(9-Fluorenylmethyloxycarbonyl)-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine (16). Compound **15** (0.633 g, 0.1 mmol) was added to a solution of **2** (0.209 g, 0.1 mmol) in a mixture of dioxan (1 mL) and 0.33 M K_2CO_3 (3 mL), and the mixture was stirred under reflux for 45 min, diluted to 200 mL with Et_2O, and extracted with 0.2 N HCl (100 mL) followed by 1 M K_2CO_3 (3 × 200 mL) and distilled H_2O (2 × 200 mL). The Et_2O layer was dried (Na_2SO_4) and evaporated, and the residue was recrystallized from EtOAc–hexanes (400 mL) containing a drop of AcOH: yield 0.477 g (60%); mp 40–42 °C; TLC R_f 0.27 (E). Anal. Calcd for $C_{39}H_{60}N_4Si_2O_9$: C, 59.66; H, 7.70; N, 7.14; Si, 7.15. Found: C, 59.82; H, 7.78; N, 7.04; Si, 7.09.

N^{α}-[N^{α}-[N^{α}-(9-Fluorenylmethyloxycarbonyl)-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine (17). A mixture of **8** (0.600 g, 1.07 mmol) and **15** (0.697 g, 1.10 mmol) in CH_2Cl_2 (20 mL) was stirred until a clear solution was obtained and was then treated dropwise with EDPA (0.188 mL). After 2 days of stirring, the solution was evaporated to dryness, and the residue was triturated with Et_2O (100 mL) and filtered. The solid was washed with 2:1 Et_2O–hexanes (15 mL) and dried in vacuo. Two additional crops were obtained by evaporation of the filtrate and cooling to −70 °C. The total yield was 0.825 g (71%): mp 46–47 °C; TLC R_f 0.54 (G). Anal. Calcd for $C_{51}H_{48}N_6Si_3O_{12}\cdot H_2O$: C, 56.95; H, 8.06; N, 7.81; Si, 7.83. Found: C, 57.07; H, 7.93; N, 8.00; Si, 7.63.

N^{α}-(9-Fluorenylmethyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysine (18). A solution of 9-fluorenylmethyl chloroformate (2.61 g, 10 mmol) in dioxan (20 mL) was added to a suspension of N^{ϵ}-(benzyloxycarbonyl)-L-lysine (2.80 g, 10 mmol) in a mixture of dioxan (20 mL) and 1 M K_2CO_3 (40 mL), and the mixture was kept at room temperature for 5 days and worked up as in the preparation of **14**: yield 3.20 g (64%); mp 60–65 °C. Anal. Calcd for $C_{29}H_{30}N_2O_6\cdot 0.25H_2O$: C, 68.69; H, 6.06; N, 5.52. Found: C, 68.78; H, 6.30; N, 5.34.

p-Nitrophenyl N^{α}-(9-Fluorenylmethyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysinate (19). *p*-Nitrophenyl chloroformate (0.52 g, 2.5 mmol) followed by EDPA (0.5 mL) was added dropwise to a stirred solution of **18** (1.10 g, 2.19 mmol) in CH_2Cl_2 (20 mL). A vacuum was applied to the septum-stoppered flask to remove CO_2, and after 25 min a catalytic portion of DMAP (25 mg) was introduced. Pumping was resumed to remove additional CO_2, and after 1.5 h at room temperature the mixture was evaporated to dryness under reduced pressure. The residue was taken up in CH_2Cl_2 (20 mL) and filtered, and the filtrate was diluted with Et_2O and cooled at 0–4 °C. The precipitate was collected, washed with ice-cold Et_2O (50 mL), and dried in vacuo at room temperature overnight: yield 1.30 g (95%); mp 107–109 °C; TLC R_f 0.24 (C). Anal. Calcd for $C_{35}H_{23}N_3O_8$: C, 67.52; H, 5.45; N, 6.75. Found: C, 67.41; H, 5.33; N, 6.74.

N^{α}-[N^{α}-[N^{α}-(9-Fluorenylmethyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine (20). Compound **19** (0.4 g, 0.6 mmol) followed by EDPA (1 mL) and a catalytic amount of DMPA (50 mg) were added to a suspension of **8** (0.325 g, 0.58 mmol) in CH_2Cl_2 (25 mL), and the mixture was stirred vigorously for 3 days and filtered. The filtrate was evaporated to dryness, and final traces of volatile materials were removed by entrainment with EtOH (3 × 50 mL). The residue was triturated with Et_2O (50 mL), using ultrasonication to facilitate dispersion, until most of the yellow color of *p*-nitrophenol was removed. The resulting solid was collected and redissolved in CH_2Cl_2 (150 mL). The solution was concentrated to a small volume, and Et_2O (150 mL) was added to precipitate a solid, which was collected, washed with Et_2O (30 mL), and dried in vacuo overnight: yield 0.532 g (87%); mp 160–165 °C; TLC R_f 0.51 (G). Anal. Calcd for $C_{53}H_{78}N_6Si_2O_{12}$: C, 60.78; H, 7.51; N, 8.02; Si, 5.36. Found: C, 60.46; H, 7.64; N, 7.78; Si, 5.61.

N^{α}-[N^{α}-[N^{α}-(9-Fluorenylmethyloxycarbonyl)-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine (21). A solution of **20** (0.408 g, 0.39 mmol) in 1:1 MeOH–AcOH was hydrogenated for 8 h in the presence of 10% Pd–C (0.144 g) in a Parr apparatus at an initial pressure of 2.5 atm. The catalyst was filtered through sintered glass, and the filtrate was evaporated. Residual AcOH was removed by coevaporation with H_2O (100 mL) at 40 °C under reduced pressure. The residue was triturated with H_2O (100 mL), and the resulting solid was collected, washed with H_2O, and dried in vacuo: yield 0.23 g (65%); mp 174–178 °C; TLC R_f 0.15 (G). Anal. Calcd for $C_{45}H_{72}N_6Si_2O_{10}\cdot 2H_2O$: C, 56.93; H, 8.07; N, 8.85; Si, 5.92. Found: C, 56.88; H, 8.08; N, 8.87; Si, 5.75.

N^{α}-[N^{α}-[N^{α}-[N^{α}-(9-Fluorenylmethyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine (22). EDPA (0.15 mL) was added to a solution of **9** (0.708 g, 0.838 mmol) and **19** (0.523 g, 0.839 mmol) in CH_2Cl_2 (25 mL), and the mixture was stirred for 2.5 days before being evaporated to dryness under reduced pressure. The residue was chromatographed on a Sephadex LH20 column (5 cm i.d. × 60 cm) with 95% EtOH as the eluent, and fractions containing only **20** according to TLC were pooled and evaporated. The residue was triturated under Et_2O with the aid of an ultrasonic bath, and the solid was collected and dried in vacuo overnight at room temperature: yield 0.673 g (57%); TLC R_f 0.22 (B); mp 155–158 °C. Anal. Calcd for $C_{65}H_{102}N_8Si_3O_{15}\cdot H_2O$: C, 58.36; H, 7.84; N, 8.38; Si, 6.30. Found: C, 58.33; H, 8.06; N, 8.36; Si, 6.52.

N^{α}-[N^{α}-[N^{α}-[N^{α}-(9-Fluorenylmethyloxycarbonyl)-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysyl]-N^{ϵ}-[[2-(trimethylsilyl)ethoxy]carbonyl]-L-lysine (23). A solution of **22** (0.559 g, 0.418 mmol) in 49:1 EtOH–AcOH was hydrogenated in a low-pressure Parr apparatus over 10% Pd–C (0.571 g) for 2.5 h at an initial pressure of 2.5 atm. The catalyst was removed by filtration through Celite, and the filtrate was evaporated to dryness under reduced pressure at room temperature. The residue was taken up in EtOH (5 mL), the solution diluted with Et_2O (60 mL) and stirred for 15 min, and the precipitate was collected and dried in vacuo. The mother liquor was concentrated to a small volume and diluted with another portion of Et_2O (60 mL) to obtain a second crop for a total yield of 0.476 g (81%): mp 110–112 °C; TLC R_f 0.18 (G). Anal. Calcd for $C_{57}H_{96}N_8Si_3O_{13}\cdot 3.5H_2O$: C,

54.82; H, 8.31; N, 8.97; Si, 6.75. Found: C, 54.92; H, 7.95; N, 9.15; Si, 6.73.

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Registry No. 1, 85167-76-6; 2, 85167-75-5; 3, 122924-32-7; 4, 122903-59-7; 5, 122903-60-0; 6, 122903-61-1; 7, 122903-62-2; 8 (free base), 122903-63-3; 8·TsOH, 122903-77-9; 8·TmbsOH, 122903-78-0; 9, 122903-64-4; 10, 34404-36-9; 11, 122903-65-5; 12, 122903-66-6; 13, 122903-67-7; 14, 122903-68-8; 15, 122903-69-9; 16, 122903-70-2; 17, 122903-71-3; 18, 86060-82-4; 19, 122903-72-4; 20, 122903-73-5; 21, 122903-74-6; 22, 122903-75-7; 23, 122903-76-8; BOC-Lys(Z)-OH, 2389-45-9; Fmoc-Cl, 28920-43-6; Fmoc-Lys-OH, 105047-45-8; H-Lys(Z)-OH, 1155-64-2.

Synthesis of Stereochemically Defined ψ[CH(alkyl)NH] Pseudopeptides[1]

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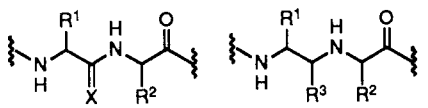
A general synthesis of ψ[CH(alkyl)NH] pseudopeptides **3**, with defined stereochemistry at the new asymmetric center, is described. Grignard reaction of amino acid derived oxazolidines **10** and **11** gave separable mixtures of benzyl-protected diamino alcohols **12–15**, the stereochemistry of which could be defined by conversion to imidazolidones **16–19**. Studies on the oxidation of these alcohols yielded a procedure for the conversion of aldehydes to acyl cyanides which is compatible with sensitive functionality. Application of this methodology to a series of monoprotected diamino alcohols (e.g. **39**) gave rise to pseudotripeptides (e.g. **41**) upon in situ coupling with (*S*)-phenylalanine methyl ester hydrochloride. This synthesis allows for variations in R^1 and R^2 of pseudopeptide **3** and permits the introduction of bulky appendages along the peptide backbone.

In recent years, considerable attention has been focused on structure–activity studies of pharmacologically interesting peptides.[2] One goal in this area deals with stabilizing a given peptide toward degradation by in vivo peptidases.[3] As a result, a variety of novel backbone-modified peptides have been synthesized in which the amide moiety of peptide **1** is replaced by groups which are inert to enzymatic hydrolysis. The synthesis of such amide bond surrogates has been reviewed[4] and includes several methods for the preparation of methyleneamino, or ψ[CH_2NH], amide bond replacements **2**. In the course of our work



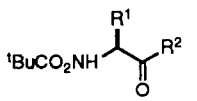
1: X = O
2: X = H_2

3

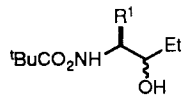
on analogues of atrial natriuretic factor, we required a general synthesis of pseudodipeptide **3** in which one of the diastereotopic protons at the original amide carbonyl carbon of **2** is replaced with an alkyl group. Pseudodipeptide **3** might therefore be designated as an alkylmethineamino amide bond replacement defined as ψ[CH(alkyl)NH] which possesses a new asymmetric carbon adjacent in most cases to another asymmetric atom (the α-carbon). Not only would amide bond replacement **3** resist proteolytic hydrolysis, but the new asymmetric center could impart unique conformational biases when incorporated into a given peptide. The synthesis of a related chiral amide bond surrogate, Glyψ[$CH(CH_3)$S], has been attempted by Spatola[5] for introduction into LH–RH derivatives; however, absent from the literature are ψ[CH(alkyl)NH] analogues with substitutions at the α-carbons on both sides of the replaced amide bond. Presented here is a general method for the synthesis of several ψ[CH(alkyl)NH] amide bond replacements of defined stereochemistry.

Results and Discussion

Methyleneamino amide bond isosteres such as **2** have been prepared efficiently by the reductive amination of protected amino aldehydes **4** with a variety of α-amino esters.[6] Our attempts to prepare pseudodipeptides **3** by reductive amination of ethyl ketone **5** (R^1 = Bn, Me) with (*S*)-valine methyl ester gave rise to a mixture of alcohols **6** under a variety of reaction conditions. This can be expected due to steric reasons, especially in cases where R^1 and R^2 effectively shield the ketone carbonyl of **5**.[7] A more general procedure which allows incorporation of bulky groups along the peptide backbone was therefore desired.



4: R^2 = H
5: R^2 = Et



6

An alternative approach is depicted in Scheme I and involves Grignard addition to amino acid derived oxazolidines, followed by oxidation of the resulting alcohols.

(1) According to IUPAC rules, the structure inside the bracket following ψ is the unit substituting for the peptide amide bond. IUPAC–IUB Joint Commission on Biochemical Nomenclature *Eur. J. Biochem.* **1984**, *138*, 9.

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