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A new silyl linker for reverse-direction solid-phase peptide synthesis

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Abstract—Treatment of a free amino acid ester with CO_2 followed by exposure to a chlorosilane-containing polystyrene results in its attachment to the solid support. The newly formed silyl carbamate can be employed to build polypeptides at the carboxyl terminus. Cleavage of the (poly)peptide using aqueous HF in CH_3CN leads to its free amine form which is isolated as a Boc derivative. The polymer support can be easily recycled.

With the advent of combinatorial chemistry, a renaissance in solid-phase organic synthesis (SPOS) is taking place. Closely associated with these advances are numerous developments in available linkers,[1] which contain various functionality and are usually modeled on solution-based protecting groups. Many linkers, therefore, are dependent upon silicon,[2] which is separated from a polymer backbone by a spacer group (Fig. 1). Cleavage of the desired product from a silyl linker frequently results from acid-induced protiodesilylation of a (substituted) aromatic ring[3] or otherwise activated silane (e.g. benzylic,[4] allylic,[5] etc.), or via net hydrolysis of a silyl ether.[6,7] Based on our recent report introducing Tsoc and its TBDPS analog as new protecting groups for nitrogen,[8a] we envisioned the carbamate within **1** functioning as a new linker which upon cleavage under the influence of fluoride ion would leave behind a hydrogen on nitrogen.[8b] We now describe this novel addition to the arsenal of SPOS, in this case as applied to unconventional *N*-linked polypeptide synthesis.[9]

Unlike traditional solid-phase peptide synthesis, an approach which proceeds in the reverse direction (i.e. N→C) leads directly to C-terminally protected/modified products.[9] Use of an *N*-bound silyl carbamate linker was anticipated to offer several advantages, including: (1) commercial availability of bromopolystyrene; (2) no requirement for a formal spacer group; (3) mild fluoride-based cleavage of the polypeptide from the resin; (4) minimal loss of stereochemical integrity throughout the sequence; and (5) ready recycling of the polymer.

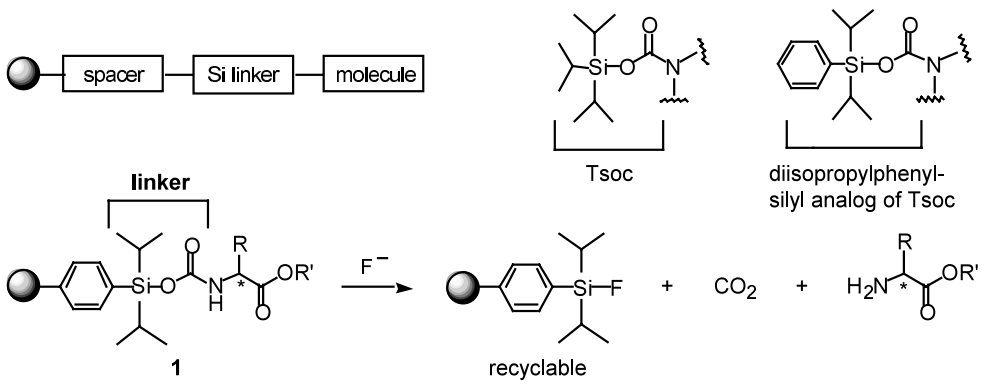

Figure 1.

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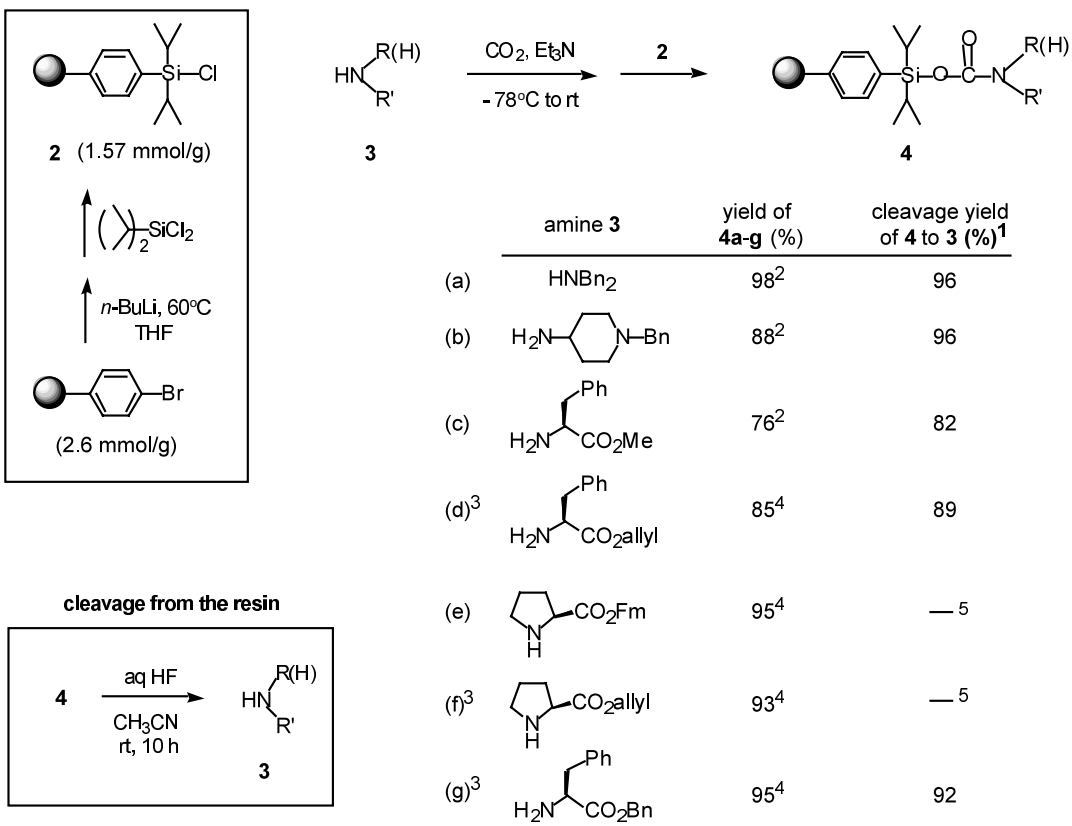
In practice, *para*-bromopolystyrene (1% crosslinked, 200–400 mesh, 2.6 mmol/g)[10] could be lithiated and quenched with $(i\text{-Pr})_2SiCl_2$[11] to afford silyl chloride **2** (Scheme 1). Upon exposure of amines **3a–g** to gaseous CO_2 in CH_2Cl_2[8a] followed by trapping of the intermediate salt with polymer-bound silyl chloride **2**, the corresponding silyl carbamates **4** were gradually formed. Release of the amine from **4** was found to occur smoothly using aqueous HF (3–4 equiv.) in CH_3CN at room temperature.[12] Among the amines mounted via the corresponding silyl carbamate, a fluorenylmethyl (Fm) ester of proline (**4e**) was found to readily undergo conversion to the free acid upon treatment with pyrrolidine in CH_2Cl_2 at room temperature. In general, however, Fm esters were very sensitive to base; hence, amino acids were best introduced as their allyl ester derivatives (e.g. **4d** and **4f**). These derivatives could be unmasked to the free carboxyl using catalytic Pd(0) in the presence of excess morpholine[13] or $Me_2NH{\cdot}BH_3$.[14] Benzyl esters were examined, and while these underwent clean removal using $Pd(0)/Et_3SiH$ in model compounds tested in solution (as their Tsoc derivatives), the polystyrene mounted analogs led to essentially no reaction.

Activation of amino acids **5** was found to be most efficient using traditional *i*-butyl chloroformate/*N*-methylmorpholine[15] (NMM; Scheme 2). Other standard conditions investigated (e.g. DCC, DMAP; HBTU, HOBT, DIPEA; HOBT, DIPCDI)[16] were less effective. Subsequent couplings with a methyl or allyl ester of phenylalanine or phenylglycine occurred smoothly. Treatment of silyl carbamates **6** with HF/CH_3CN followed by $(Boc)_2O$ led to fully protected dipeptides **7a–c** in good overall isolated yields from **4d–f**, the optical rotation of each indicating that no loss in stereochemical integrity had occurred throughout the sequence.

The two-step cycle (i.e. ester cleavage, then coupling) could be applied twice to educts **4d** and **4f** (Scheme 3), giving rise to polymer-bound tripeptide allyl esters **8**, **10**, and **12**. Final release from the polymer and *N*-protection afforded *N*-Boc tripeptide esters **9**, **11**, and **13** in good overall yields from the starting carbamates (**4d** and **4f**). The stereopurity of each product was excellent based on comparisons with authentic materials.[17] Noteworthy is the observation that incorporation of a phenylglycine residue was easily accommodated without losses due to epimerization.

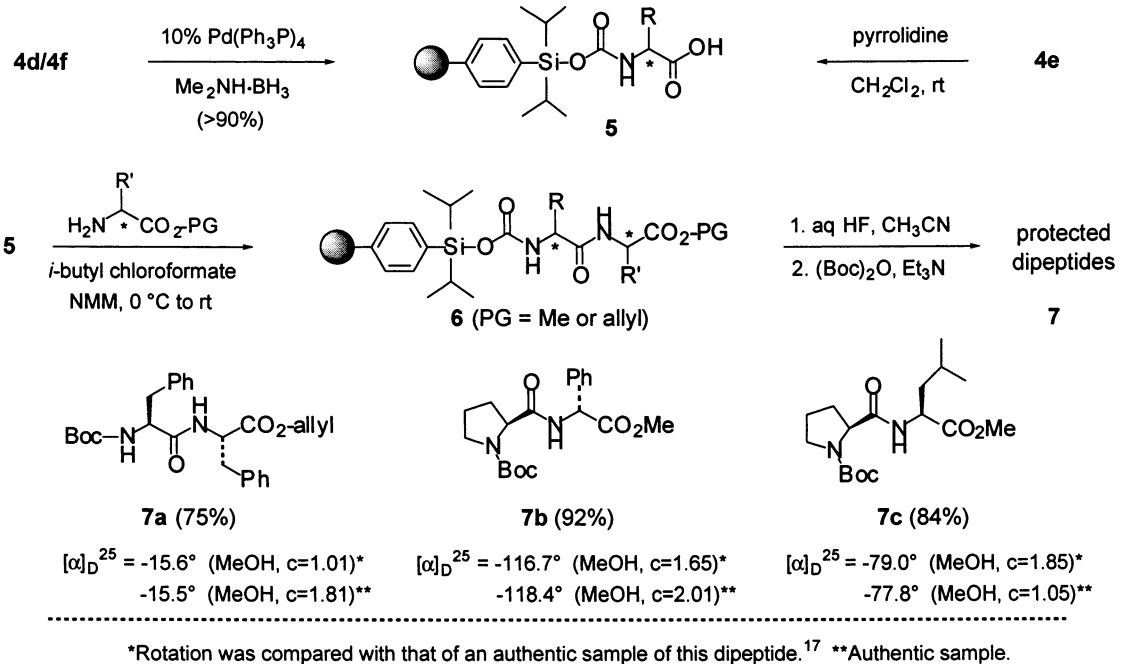
By way of comparison, the diphenylsilyl analog **14** of diisopropylsilyl carbamate **4d** was tested (Scheme 4), using the same sequence as depicted in Scheme 3 (i.e. acid deprotection and then coupling, done twice, then cleavage from the resin and *N*-Boc protection). Overall, the yield of tripeptide **9** was 15% lower, suggesting that



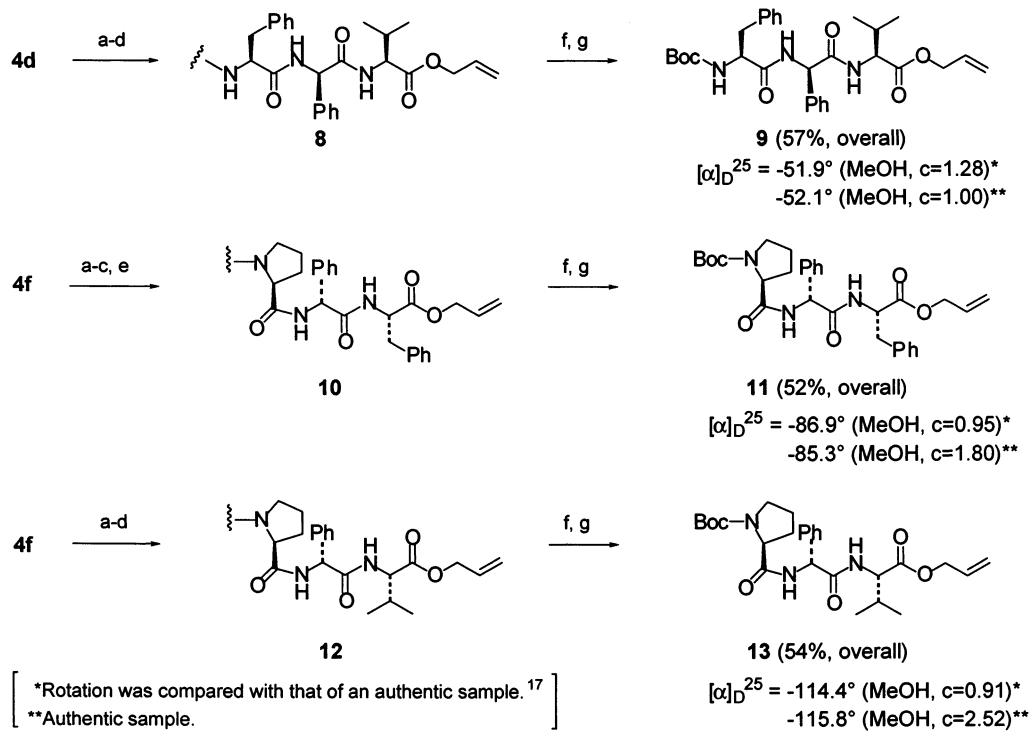
	amine **3**	yield of **4a-g** (%)	cleavage yield of **4** to **3** (%)[1]
(a)	$HNBn_2$	98[2]	96
(b)	H_2N–(piperidine)–N–Bn	88[2]	96
(c)	Ph, H_2N, CO_2Me	76[2]	82
(d)[3]	Ph, H_2N, CO_2allyl	85[4]	89
(e)	CO_2Fm	95[4]	—[5]
(f)[3]	CO_2allyl	93[4]	—[5]
(g)[3]	Ph, H_2N, CO_2Bn	95[4]	92

[1]Based on isolated material. [2]Determined by elemental analysis. [3]This example involved more highly loaded bromopolystyrene (4 mmol/g). [4]Determined by resin gain in weight. [5]Not determined.

Scheme 1.



Scheme 2.

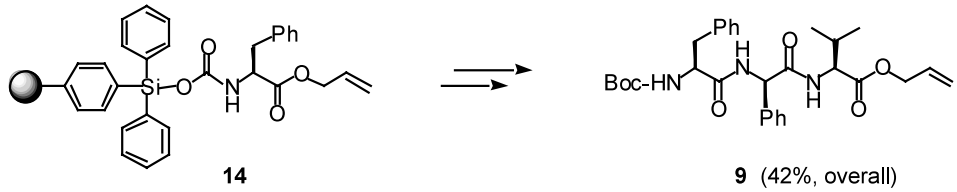


[a] cat Pd(0), $Me_2NH{\bullet}BH_3$; [b] $ClCO_2$-*i*-Bu, phenylglycine allyl ester; [c] same as in [a]; [d] valine allyl ester; [e] phenylalanine allyl ester; [f] HF, CH_3CN; [g] $(Boc)_2O$, Et_3N.

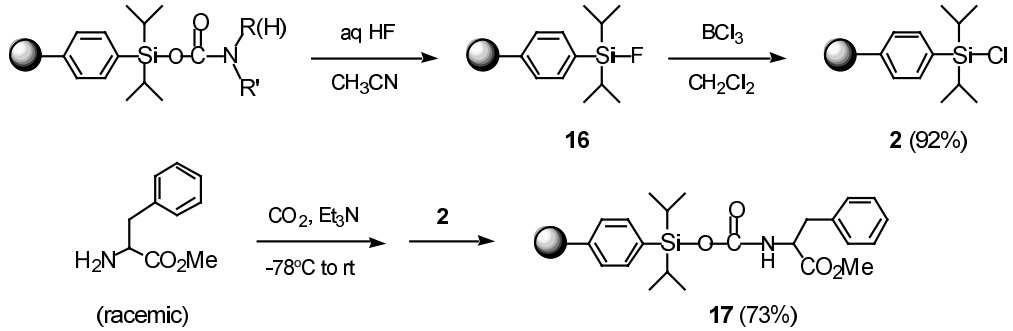
Scheme 3.

this derivative offers no obvious advantage over the diisopropyl-substituted case (cf. overall yield for the conversion of **4d** to **9** in Scheme 3).

Release of a (poly)peptide from the resin using HF led to the derived silyl fluoride **16**, which can be efficiently recycled upon treatment with BCl_3 in CH_2Cl_2 at room temperature (Scheme 5).[18] Exposure of the carbamate salt derived from phenylalanine methyl ester to regenerated resin **2** gave the expected carbamate-derived product **17**.



Scheme 4.



Scheme 5.

In summary, a novel silyl carbamate linker[19] has been developed which has been demonstrated for reverse (N→C) direction solid-phase peptide synthesis. Overall efficiencies are good, and the purities of the final products are excellent. Cleavage of the free amine moiety from the resin occurs under mild fluoride-mediated conditions, leaving behind only CO_2 as by-product. That the solid support can be readily recycled to the active silyl chloride is a particularly noteworthy feature. Other uses of this silyl linker in SPOS involving amines are envisioned and will be reported in due course.

Acknowledgements

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19. *General procedure for the preparation of polymer-bound, silyl carbamate-linked amines* ***4****: carbamate* ***4b***: To a solution of 4-amino-1-benzyl-piperidine (0.60 g, 3.14 mmol) in dry CH_2Cl_2 (5 mL) was added Et_3N (0.42 mL, 3.14 mmol) at −78°C. CO_2 gas was bubbled into the mixture at −78°C for 1 h. In another flask was placed **2** (1.00 g, 1.57 mmol) suspended in dry CH_2Cl_2 (5 mL) and cooled to −78°C. The reaction mixture was cannulated into the suspension of **2** at −78°C and stirred for 2 h at this temperature. The reaction mixture was then gradually warmed to rt and stirred for an additional 10 h. The resin was then filtered and washed sequentially with CH_2Cl_2 (100 mL), DMF (100 mL), 50% H_2O/DMF (100 mL), DMF (100 mL) and CH_2Cl_2 (100 mL). Drying under vacuum (ca. 5 mmHg) for 24 h afforded **4b** (1.2 g, 1.06 mmol/g) in 88% yield, based on analytical data. Anal. calcd: N, 3.40; Si, 4.77. Found: N, 2.96; Si, 4.20. IR (KBr) 3400, 2901, 1700, 1683, 1511, 1334, 1107, 687 cm^{-1}. *Representative sequence of reactions: conversion of* ***4f*** *to protected dipeptide* ***7b***: To a suspension of **4f** (0.5 g, 0.65 mmol) in anhydrous CH_2Cl_2 (5 mL), was added dropwise at rt a solution of $(CH_3)_2NH{\cdot}BH_3$ (231 mg, 3.9 mmol) in CH_2Cl_2 (2 mL). After stirring for 10 min, a solution of $Pd(PPh_3)_4$ (74.9 mg, 0.065 mmol) in CH_2Cl_2 (2 mL) was added at the same temperature. After stirring for 2 h, the resin was filtered and washed with CH_2Cl_2 (50 mL), DMF (50 mL), 50% DMF in water (50 mL), DMF (50 mL) and CH_2Cl_2 (50 mL), sequentially. The resin was dried under vacuum for 24 h, and then suspended in anhydrous CH_2Cl_2 (10 mL) to which was added isobutyl chloroformate (421 μL, 3.25 mmol) and *N*-methylmorpholine (361 μL, 3.25 mmol) at 0°C. The reaction mixture was stirred for 2 h at this temperature and then warmed to rt. After stirring for an additional 2 h, the resin was filtered and washed with anhydrous CH_2Cl_2 (20 mL) under Ar. The resin was suspended in anhydrous CH_2Cl_2 (5 mL) and a solution of D-phenylglycine methyl ester (536 mg, 3.25 mmol) in CH_2Cl_2 (1 mL) was added at 0°C. The reaction mixture was stirred 3 h at 0°C and then warmed to rt. After stirring for an additional 12 h at rt, the resin was filtered and washed with CH_2Cl_2 (50 mL), DMF (50 mL), 50% DMF in water (50 mL) and finally CH_2Cl_2 (50 mL). The resin was dried under vacuum for 12 h and then suspended in CH_3CN (5 mL) to which was slowly added 49% aqueous HF solution (79.6 μL, 1.95 mmol). Stirring was continued for 5 h at rt after which Et_3N (453 μL, 3.35 mmol) was added at 0°C. After raising the temperature to rt, the resin was filtered off and washed with additional CH_2Cl_2 (10 mL). The combined filtrate was concentrated in vacuo and the crude dipeptide dissolved in CH_2Cl_2 (5 mL) to which was added $(Boc)_2O$ (121 mg, 0.98 mmol) and Et_3N (136 μL, 0.98 mmol) at 0°C. After stirring for 1 h, the reaction mixture was warmed to rt with stirring for 3 h. The resin was then filtered off and washed with CH_2Cl_2 (20 mL). The combined filtrate was washed with H_2O (20 mL), dried over $MgSO_4$, and concentrated under vacuum. Purification over a silica gel (EtOA/hexanes = 2/1, $R_f = 0.61$) afforded the desired product **7b** as a white solid (216 mg, 92%); $[\alpha]_D^{25} = -116.7°$ (*c* 1.65, MeOH); IR (KBr) 3339, 2972, 1747, 1649, 1532, 1390, 984, 701 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.17–7.20 (m, 5H), 5.06 (m, 1H), 4.25 (m, 1H), 3.78 (s, 3H), 3.50 (m, 2H), 1.95 (m, 2H), 1.20 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.2, 172.5, 171.1, 136.4, 128.9, 128.5, 127.2, 80.5, 66.4, 56.2, 47.1, 30.1, 24.1; HRMS (FAB) calcd for $C_{19}H_{26}N_2O_5$ $(M+Na)^+$ 385.1739, found 385.1736.