The (2-Phenyl-2-trimethylsilyl)ethyl-(PTMSEL) Linker—A Novel Linker for the Solid-Phase Synthesis of Protected Peptides and Glycopeptides Cleavable with Fluoride**

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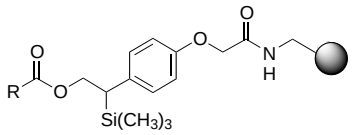
Dedicated to Professor Lutz F. Tietze on occasion of his 60th birthday

Synthesis on solid phase is of great importance for the rapid access to peptides and peptide conjugates.[1] In particular this holds true for the combinatorial synthesis of compound libraries and large groups of single compounds. In the meantime, the principle of combinatorial synthesis has been expanded to the generation of many different classes of organic compounds.[2]

In all variations of combinatorial solid-phase syntheses, the linker is of crucial importance. It must be stable throughout the multistep synthesis, but finally should be cleavable under mild conditions without affecting the products. Most commonly used in solid-phase peptide synthesis are acid-labile linkers,[1, 2] such as the *p*-alkyloxybenzyl ester and triphenylmethyl(trityl, Trt) ester type linkers.[3] During their cleavage, acid-labile protecting groups, for example in the amino acid side chains of the synthesized peptides, can be removed simultaneously. Under acidic and basic cleavage conditions, undesired side reactions often take place in the produced peptides, among which, aspartimide formation and rearrangements in aspartyl peptides are very difficult to prevent.[4, 6] Most of these side reactions can be avoided by using linker systems, which are cleavable under neutral conditions such as the allylic anchors.[5] Because of their insufficient steric hindrance, the allyl-ester type linkers are sometimes susceptible to aminolysis. Accordingly, they are prone to diketopiperazine formation at the stage of the polymer-bound dipeptides after removal of the 9-fluorenylmethoxycarbonyl(Fmoc) group as amino protecting group.

p-Silylmethylbenzyl,[7] *p*-silyloxybenzyl[8] and α-trimethylsilylbenzyl (SAC)[9] linkers based on the (2-trimethylsilyl)ethyl (TMSE) ester[6a] and cleavable with fluoride ions represent a further option for the mild detachment of peptides from the polymer support. However, even the most sensitive among them, the SAC linker,[9] needs tetrabutylammonium fluoride (TBAF) trihydrate in dimethylformamide (DMF) for its cleavage, which for a tetrapeptide results in accompanying aspartimide formation of up to 80%. In contrast, when benzyltrimethylammonium hydrogen difluoride in DMF was used as the cleaving reagent, the cleavage yield in the synthesis of two simple tetrapeptides was unsatisfactory (30% – 40%), and the crude products still contained up to 10% of the aspartimide. Therefore, these authors recommend the acidolysis with 1% trifluoroacetic acid (TFA) in dichloromethane for the cleavage of their SAC linker, especially since the Fmoc group is stable under these cleavage conditions, but not during the fluoridolysis using TBAF in DMF.[9, 10]

The (2-phenyl-2-trimethylsilyl)ethyl(PTMSEL) linker **1** represents a novel anchor for solid-phase peptide synthesis using Fmoc chemistry. It is based on the (2-phenyl-2-trimethylsilyl)ethyl (PTMSE) protecting group[11] and renders the liberation of protected, especially Fmoc-protected peptides and glycopeptides in form of the C-terminally free carboxylic acids. In contrast to the SAC linker **2**,[9] in the



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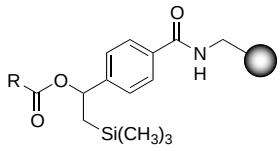

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PTMSEL linker not the C–O bond is benzylic and thereby weakened, but the C–Si bond. As a consequence the PTMSEL linker is distinctly more sensitive towards fluoridolysis and more stable towards acidolytic conditions. Thus, its cleavage was achieved with TBAF · 3 H_2O in CH_2Cl_2 under almost neutral conditions (Scheme 1)

To synthesize the PTMSEL linker (Scheme 2), trimethylvinylsilane (**3**) was epoxidized with *m*-chloroperbenzoic acid (mCPBA) to give trimethylsilyloxirane (**4**). Reaction of **4** with lithium di[(*p*-1-ethoxyethyloxy)phenyl]cuprate, which is generated in situ by lithiation of 1-(4-bromophenoxy)-1-ethoxyethane with *n*-butyllithium and subsequent reaction with copper(I) iodide, efficiently yields (2-*p*-ethoxyethyloxyphenyl-2-trimethylsilyl)ethanol (**5;** 85%). The ethoxyethyl(EE) protecting group is removed in MeOH under catalysis with pyridinium-*p*-toluenesulfonate (PPTS). The PTMSEL linker, (2-*p*-hydroxyphenyl-2-trimethylsilyl)ethanol (**6**), is obtained in a yield of 90% (76% from **4**).

(2-*p*-Hydroxyphenyl-2-trimethylsilyl)ethanol is linked to the polymer support through its phenolic OH group (Scheme 2). The *N*-acyl amino acid is coupled to the linker molecule in solution. Esterification on solid phase is often accompanied by racemization. The alcohol **6** reacts with allyl chloroacetate to give allyl 4-[2-hydroxy-1-(trimethylsilyl)-ethyl]phenoxy acetate (**7**). Acylation with the Fmoc-protected amino acid (Fmoc-AA-OH) using dicyclohexylcarbodiimide (DCC) and catalytic amounts of 4-dimethylaminopyridine (DMAP)[12] gives the allyl ester protected compound **8** (yields: > 90%). The allyl ester of **8** is removed quantitatively by using [$Pd(PPh_3)_4$] and sodium *p*-toluenesulfinate[13] or another allyl trapping reagent[14] leading to acid **9** (yield ca. 54% over five steps starting from **4**). Acid **9** can be linked to an amino-functionalized resin by using *O*-(benzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluroniumtetrafluoroborate (TBTU)/*N*-hydroxybenzotriazole (HOBt)/diisopropylethylamine (DIPEA)[15] in DMF/CH_2Cl_2. Aminomethylpolystyrene (AMPS; ACT; 200 – 400 mesh; loading: 1.00 mmol g^{-1}) or amino-functionalized TentaGel[16] (Nova Syn Tg amino resin;

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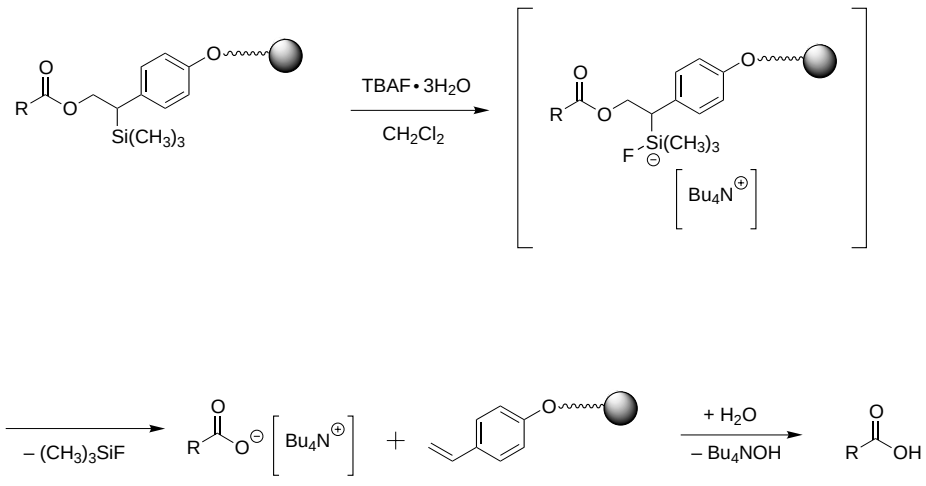
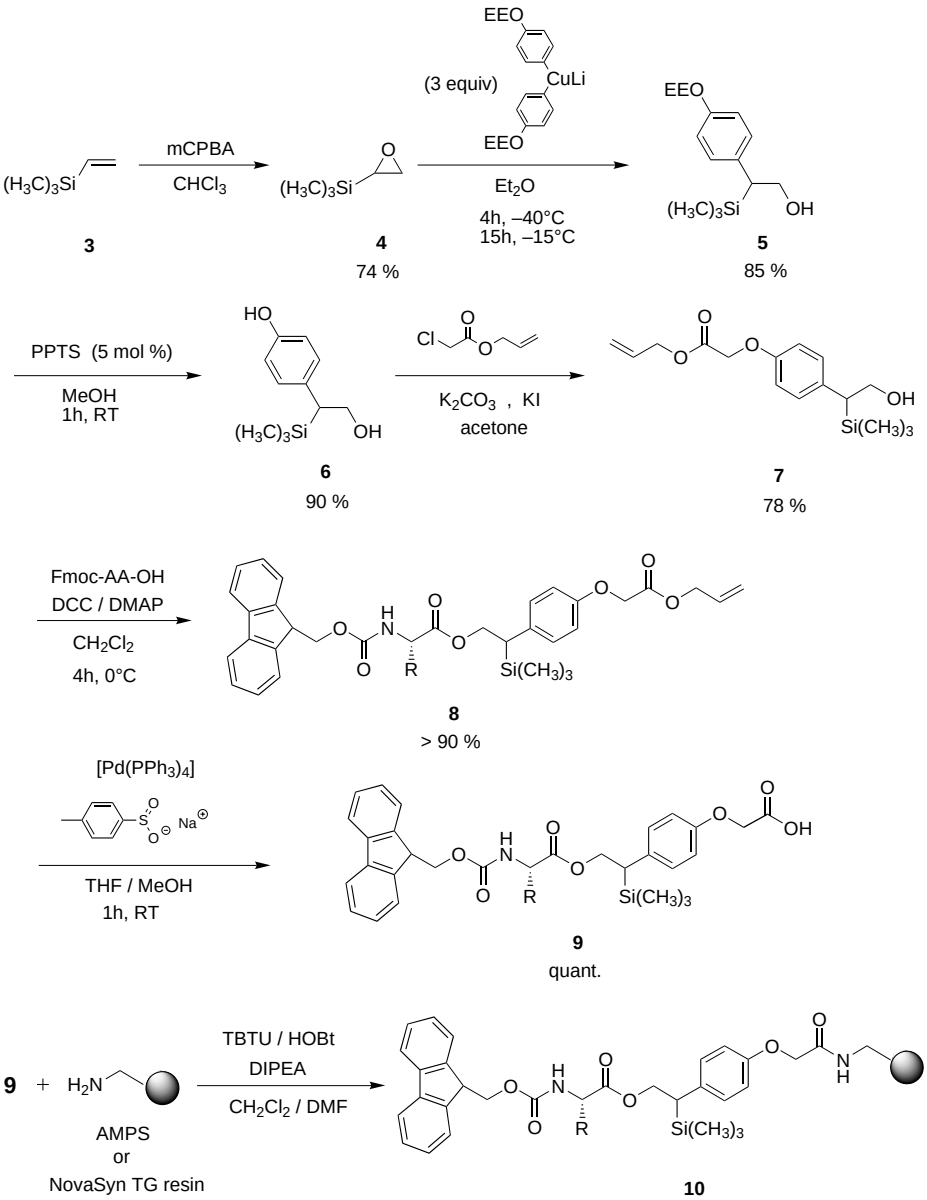


Scheme 1. Fluoride-induced cleavage of the PTMSEL linker.



Scheme 2. Synthesis of the PTMSEL linker and attachment of the first amino acid.

Novabiochem, 110 μm beads, loading: 0.43 mmol g^{-1}) were used as polymer supports. Unreacted amino groups were capped with acetanhydride/pyridine (1:3).

The advantageous properties of the PTMSEL linker are demonstrated in the demanding synthesis of a T_N-antigen glycodecapeptide from the extracellular domain EC1 of LI-cadherin[17] (rat), an important cell adhesion protein, which is predominantly found on liver or intestinal cells. Starting from polymer **10a** loaded with Fmoc-Ile-OH (loading: 0.365 mmol g^{-1}), glycodecapeptide Boc-Leu-Ala-Ala-Leu-Asp(O*t*Bu)-Ser(αAc$_3$GalNAc)-Gln(Trt)-Gly-Ala-Ile-OH (**11**; Boc = *tert*-butoxycarbonyl) was assembled with a peptide synthesizer (Perkin-Elmer ABI 433 A). (Scheme 3) Fmoc deprotections were carried out by up to five four-minute treatments with 30% piperidine in *N*-methylpyrrolidone (NMP) and peptide couplings by reaction with 10 equivalents of Fmoc- or Boc-protected amino acid and coupling reagents (HBTU, HOBt, DIPEA) within 35 minutes. The glycosylated building block Fmoc-Ser(αAc$_3$GalNAc)-OH[18] (3 equiv) was coupled manually using *O*-(7-aza-benzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU)/*N*-hydroxy-7-azabenzotriazole (HOAt).[19] After each coupling unreacted amino groups were capped with acetanhydride/DIPEA/HOBt. After completion of the peptide synthesis, the resin was extensively washed with NMP and CH_2Cl_2, which is important because NMP or DMF must be completely removed before cleaving the PTMSEL linker with tetabutylammonium fluoride. TBAF · 3 H_2O is a strong base in polar, aprotic solvents such as DMF, NMP, or THF, but only a weak base (almost neutral) in CH_2Cl_2. The fluoride-induced detachment from the polymer support was achieved manually by shaking the resin with two equivalents of TBAF · 3 H_2O (0.2 mmol) in CH_2Cl_2 for 25 min.[20] Figure 1 shows the chromatogram (HPLC) of crude product **11**, which was obtained after cleavage of the PTMSEL linker.

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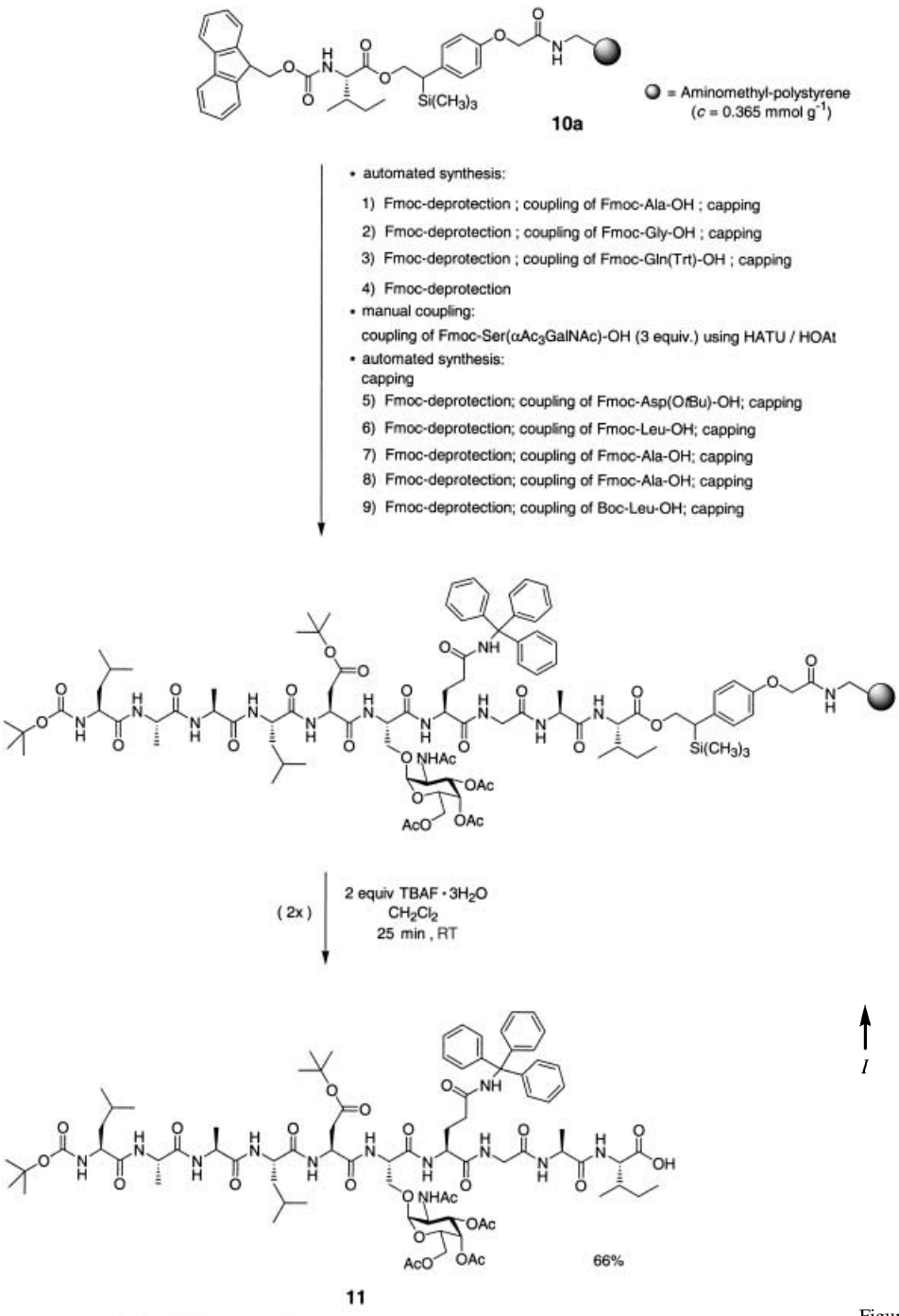


Scheme 3. Synthesis of a glycodecapeptide from LI-cadherin (rat).

Compounds were identified by HPLC-MS analysis. The major peak (92%) corresponds to the desired product **11**. As side products the aspartimide-containing peptide **12** (2%) and products from various deletion sequences (together $\approx$4%) were identified. β-Elimination at the glycosylated serine building block or deacetylation were not observed. Purification was performed by preparative HPLC, which gave glycodecapeptide **11** in 66% yield with respect to the loading of the polymer **10a**.[21] Two properties are remarkable: a) In contrast to other linkers, which need to be cleaved with TBAF in DMF, NMP, or THF, side reactions such as the formation of aspartimides or rearranged aspartyl peptides are extensively suppressed during the mild cleavage of the PTMSEL linker. (During the incomplete cleavage of the SAC linker **2** with benzyltrimethylammonium hydrogen difluoride (BTAHF), aspartimide formation is also suppressed.[9] But it is not clear, whether this "super acid labile" linker (1% TFA in CH_2Cl_2) is cleaved by fluoride and not by the half-neutralized hydrofluoric acid.) b) During the cleavage of the PTMSEL linker most of the common protecting groups in peptide chemistry (even Fmoc, see Scheme 4) and also base-labile glycosidic bonds and protecting groups in glycopeptide syntheses remain stable. Thus, protected glycopeptides can be detached from solid phase and directly used for fragment condensations.

The PTMSEL linker is sterically so demanding that during the removal of the Fmoc group no loss of the polymer-bound dipeptide occurred. Such side reactions often take place in Pro-Pro-linker-, Tyr-Pro-linker-

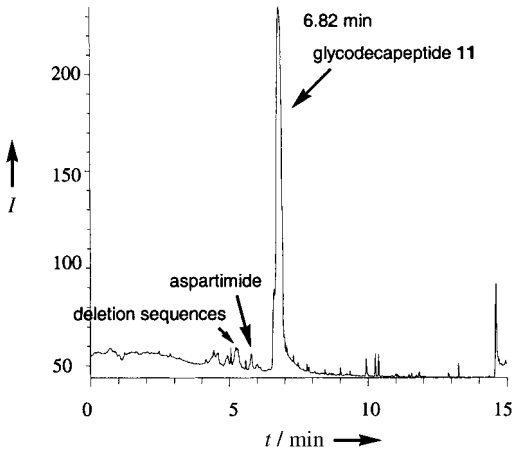
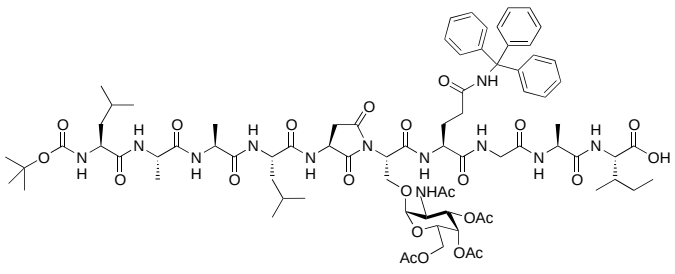


Figure 1. Chromatogram (HPLC) of the crude glycopeptide **11** directly after cleavage from the solid support.[21]



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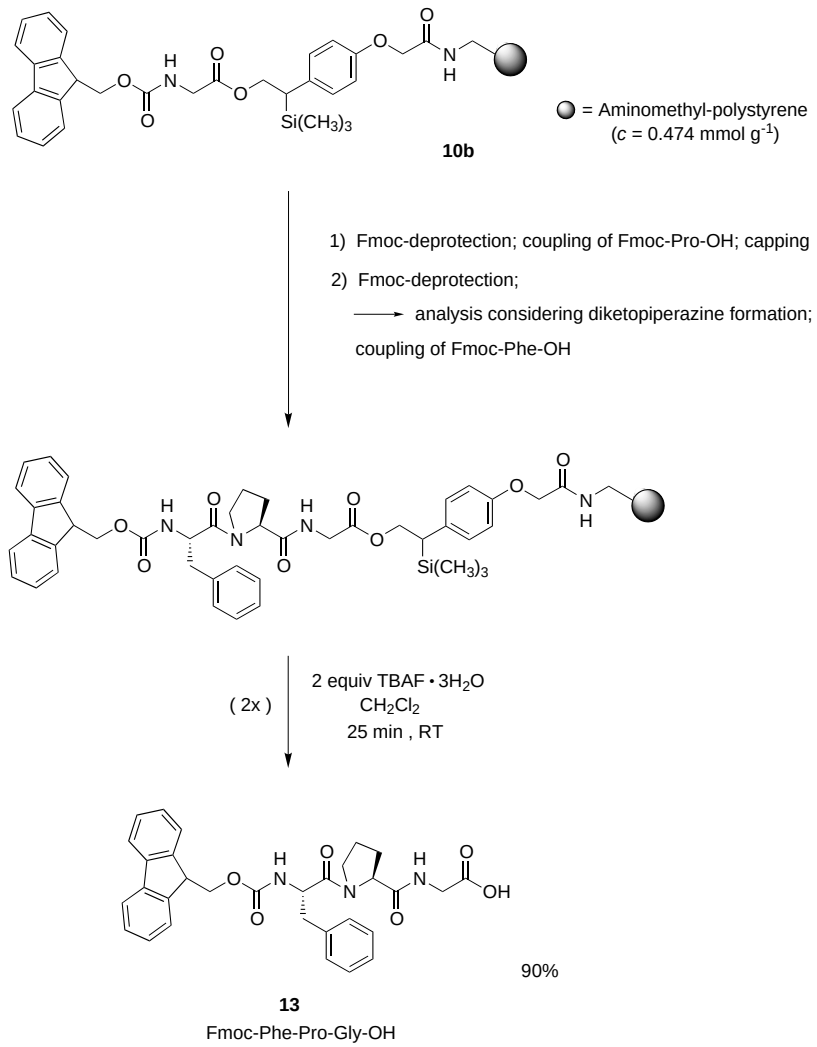

Scheme 4. Synthesis of a Fmoc-protected tripeptide containig a Pro-Gly sequence, usually prone to diketopiperazine formation.

and Pro-Gly-linker-sequences. The solid-phase synthesis of tripeptide Fmoc-Phe-Pro-Gly-OH (**13**) is shown in Scheme 4. Even for this sequence, diketopiperazine formation was not observed, which was proven by loading analysis after each coupling as well as by HPLC-MS analysis of the filtrate obtained from the Fmoc deprotection from the second amino acid (Pro). Usual column chromatography gave the desired *N*-terminal Fmoc-protected tripeptide **13** in 90% yield with respect to the loading of **10b**.

Thus, the PTMSEL linker is particularly useful for the solid-phase synthesis of protected peptides and glycopeptides using Fmoc chemistry. Release from the solid support is simply achieved without expensive chemicals, yielding almost pure peptides. Most of the common protecting groups (Fmoc, Boc, benzyloxycarbonyl (Z), allyloxycarbonyl (Aloc), *tert*-butyl, benzyl, allyl, trityl, acetyl, etc.) are stable under the mild cleavage conditions of the PTMSEL linker, thus providing the possibility of orthogonal, three-dimensional protecting group strategies. Aspartimide formation, which is often a problem in solid-phase peptide synthesis, is decisively suppressed. With base-labile *O*-glycopeptides, side reactions do not take place. The new linker is sterically so demanding that diketopiperazine formation at the stage of the polymer-bound dipeptide is prevented.

Solid-phase synthesis using Boc chemistry is not possible, because the PTMSEL linker is cleaved by treatment with TFA. This lability has already been described for the PTMSE-protecting group.[11]

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[20] Cleavage procedure: The resin was shaken with two equivalents of TBAF · 3H$_2$O (0.2 mmol) in CH_2Cl_2 (15 mL) for 25 min. After filtration, the resin was washed with CH_2Cl_2 (3 × 10 mL). Combined filtrates were extracted with H_2O (10 mL) to remove remaining fluoride. The cleavage protocol was repeated with TBAF · 3H$_2$O (ca. 0.7 equiv, 0.07 mmol, 2 equiv with respect to the residual product on the resin). The combined CH_2Cl_2 solutions were washed twice with

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water, dried over $MgSO_4$, and concentrated in vacuo. The protected peptide was obtained in an almost pure form.

[21] $R_t = 6.82$ min (Luna C18-2/75 × 4.6 mm, 3 μm, 1 mL min^{-1}; A: H_2O, B: CH_3CN; 0–0.75 min: 20% B in A, 0.75–10 min: 20% B in A to 100% B, 10–12.5 min: 100% B, 12.5–13.5 min: 100% B to 20% B in A, 13.5–15 min: 20% B in A); $[\alpha]_D^{23} = 1.96$ ($c = 0.90$ in MeOH); ^{1}H NMR (400 MHz, [D$_6$]DMSO, ^{1}H,^{1}H-COSY): $\delta = 8.53$ (s_b, 1H; ω-NH Q); 8.08–8.14 (m, 3H; NH D, S, A); 7.85–7.98 (m, 5H; NH 2 × A, Q, L, G); 7.61 (d, 1H; NH GalNAc, $J = 8.6$ Hz); 7.14–7.26 (m, 15H; Trt); 6.85 (d, 1H; NH-urethane, L, $J = 7.43$ Hz); 5.28 (s, 1H; H-4′); 5.00 (dd, 1H; H-3′, $J_{H3,H4} = 2.74$ Hz, $J_{H3,H2} = 11.74$ Hz); 4.85 (d, 1H; H-1′, $J_{H1,H2} = 3.13$ Hz); 4.56–4.64 (m, 1H; D$^\alpha$); 4.47–4.52 (m, 1H; S$^\alpha$); 3.94–4.40 (m, 9H; 3 × A$^\alpha$, I$^\alpha$, Q$^\alpha$, L$^\alpha$, H-2′, H-5′, H-6′); 3.88–3.92 (m, 2H; L$^\alpha$, H-6′); 3.72–3.76 (m, 2H; G$^\alpha$); 3.60–3.64 (m, 2H; S$^\beta$); 2.71–2.73 (m, 1H; D$^\beta$); 2.45–2.49 (m, 1H; D$^\beta$); 2.32–2.38 (m, 2H; Q$^\chi$); 2.07 (s, 3H; CH_3 NHAc); 1.71–1.94 (m, 12H; 3 × CH_3 OAc, I$^\beta$, Q$^\beta$); 1.52–1.59 (m, 2H; L$^\chi$); 1.36 (s, 9H; 3 × CH_3 Boc); 1.33 (s, 9H; 3 × CH_3 *t*Bu); 1.33–1.42 (m, 5H; 2 × L$^\beta$, I$^\chi$); 1.11–1.18 (m, 10H; 3 × A$^\beta$, I$^\chi$); 0.79–0.86 (m, 18H; 4 × L$^\delta$, I$^\gamma$, I$^\delta$); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO, broad band, DEPT): $\delta = 172.86, 172.69, 172.18, 172.11, 172.02, 171.53, 170.39, 170.19, 170.04, 169.97, 169.91, 169.71, 169.34, 169.05, 168.22, 168.11, 156.03$ (C=O); 145.00 (C_{ipso}-Trt); 128.61 (C_{para}-Trt); 127.54 (C_{ortho}-Trt); 126.41 (C_{meta}-Trt); 97.29 (C-1′); 80.39 (C_{quart} Boc); 78.34 (C_{quart} *t*Bu); 69.37 (C_{quart} Trt); 67.51 (C-3′); 66.96 (S$^\beta$); 66.83 (C-4′); 65.92 (C-5′); 61.14 (C-6′); 56.04 (I$^\alpha$); 52.66, 52.39, 52.20 (2 × L$^\alpha$, S$^\alpha$); 50.87, 49.28, 48.08, 47.95, 47.59, 46.57 (D$^\alpha$, Q$^\alpha$, 3 × A$^\alpha$, C-2′); 41.61 (G$^\alpha$); 40.37, 40.22 (2 × L$^\beta$); 36.92 (D$^\beta$); 36.08 (I$^\beta$); 32.39 (Q$^\chi$); 27.89 (3 × CH_3 Boc); 27.66 (Q$^\beta$); 27.37 (3 × CH_3 *t*Bu); 24.46 (I$^\chi$); 23.94, 23.82 (2 × L$^\chi$); 22.69, 22.33, 21.21, 20.22 (4 × CH_3 acetyl); 20.18, 20.13 (4 × L$^\delta$); 18.05, 17.67, 17.38 (3 × A$^\beta$); 15.24 (I$^\chi$); 11.08 (I$^\delta$); MALDI-TOF-MS (α-cyanocinnamic acid matrix, positive-ion mode): m/z: 1709.3 $[M+Na]^+$, 1725.2 $[M+K]^+$, 1731.3 $[M+2Na]^+$.