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A New Highly Versatile Handle for Chemistry on a Solid Support: The Pipecolic Linker

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Abstract: The design, synthesis, and potential application of the pipecolic linker is presented. This new versatile handle can immobilize primary, secondary, and aromatic amines, as well as alcohols, phenols, and hydrazides, on a solid support. Compared with other linkers, the anchoring step is easy and efficient. The release of final products from the resin proceeds upon acidic treatment with high purities. The pipecolic linker offers the promise of being using in peptide chemistry to produce peptides modified at the N and C terminus, peptidomimetics, as well as small organic molecules.

Keywords: amino acids · C-terminal modification · hydrazides · pipecolic linkers · solid-phase synthesis

Introduction

The synthesis of several biologically active compounds (e.g., peptides and peptidomimetics) involves pathways originating with the anchoring of amine and alcohol groups to a solid support.[1] A search for new, potent, peptide-derived compounds, as well as for synthetic intermediates for either bioconjugation or ligation often necessitates modifications at the C terminus[2] to introduce functional groups such as alcohols, ethers, esters, thioesters,[3] N-alkylamides, aldehydes, hydrazides, or even to obtain cyclic molecules. To address these issues, several strategies involving attachment of the C terminus to a linker and its further release by a nucleophilic attack to give a desired functional group at the end of the synthesis were introduced.[4] Three alternative approaches leave the C terminus open to chemical modifications: secondary amide backbone anchoring,[5–8] N-terminus anchoring,[9,10] or side-chain anchoring. In the last case, each functional group on a side chain (e.g., the guanidine group of arginine,[11] the alcohol[12] of serine and threonine, the ε-amino group of lysine) requires an appropriate linker. Acid-labile linkers are currently probably the most widely used with regards to straightforward cleavage and post-cleavage workups, for example, simply removing the trifluoroacetic acid (TFA) cocktail by evaporation. However, commercially available, acid-labile linkers are not so common for the efficient anchoring and releasing of amine- or alcohol-containing compounds. Hindered trityl-related linkers, such as 2-chloro- or 4-carboxychlorotrityl linkers, map out a direct route for the anchoring of a wide variety of nucleophiles. Nevertheless, the loading efficiency significantly decreases when bulky or nonreactive nucleophiles (e.g., aromatic amines or alcohols) are used. Moreover, trityl-based linkers are very sensitive to acidic treatment, which can be a disadvantage when mild acidic conditions are required throughout the solid-supported synthetic route of target compounds. A carbamate or a carbonate linkage can be an alternative way to the direct immobilization of amines or alcohols, but it requires derivatization of the alcohol linker and the loading yields are usually very low. Apart from the above-described disadvantage, the carbamate linker is not always stable in the case of a nucleophilic attack.[10]

The above considerations prompted us to develop a versatile linker suitable for immobilization of electron-rich moieties such as amines, alcohol, and hydrazines. We propose the

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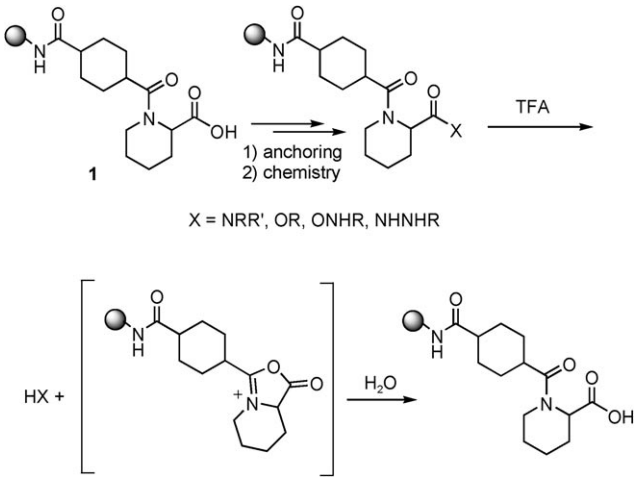
concept of a novel TFA-labile linker based on a pipecolic acid scaffold (Scheme 1).



Scheme 1. The use of the pipecolic linker anchored on the amino methyl polystyrene (PS) resin.

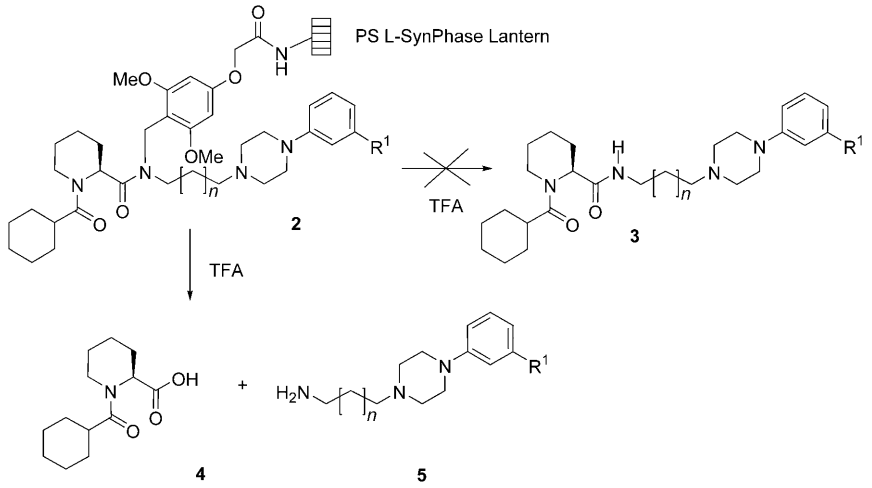
The carboxylic acid functional group of pipecolic acid can be readily activated to anchor a variety of nucleophiles. Its usefulness has been demonstrated for the anchoring of either an alcohol and amine side chain, or the N terminus of peptides and pseudopeptides, as well as for the preparation of C-terminal peptide hydrazides. These modifications are of great importance for hydrazone chemical ligation strategies,[13] or as a precursor of azide-activated peptides.[14]

Results and Discussion

Linker design: The pipecolic (Pip) linker was designed on the basis of a side reaction observed on 4-(4-formyl-3,5-dimethoxyphenoxy)butyryl linker (BAL)-linker-functionalized SynPhase Lanterns, while synthesizing a focused library of long-chain arylpiperazines.[15] Surprisingly, the TFA cleavage of the Lantern-bound pipecolic acid derivatives **2** did not yield the desired *N*-acylpipecolyl amides **3**. TFA treatment caused an unprecedented selective hydrolysis of the linker-bound amide bond, which yielded two unexpected products: the free primary amine **5** and the *N*-acylated pipecolic acid **4** (Scheme 2).

It is noteworthy that the amide bond hydrolysis also took place with acyl substituents other than cyclohexanecarbonyl (e.g., adamantanecarbonyl, 2-norbornaneacetyl). Moreover, no hydrolysis of the amide bond was observed when pipecolic acid (a six-membered ring) was replaced with its five-membered analogue, proline, which reinforces the hypothesis that a structural feature of pipecolic acid was responsible for the cleavage. Unusual acid cleavage reactions were previously reported for derivatives containing N-alkylated residues; the amide bond linking the N-Me-Aib to the subsequent amino acid was cleaved.[16] Interestingly, electron-donating substituents increased the cleavage rate, whereas electron-withdrawing substituents slowed down the process. The hydrolysis of pipecolic acid derivatives was described by a few authors,[17–20] of whom Wei et al.[17] reported an unexpected cleavage of urea-containing pipecolic acid derivatives. Maison et al.[18] proposed a mechanism of cyclohexenamide hydrolysis involving oxazolinium-5-one as an intermediate. A similar mechanism was recently postulated describing TFA-catalyzed cleavage reaction for peptides containing pipecolic acid residues.[19,20] On the basis of the above-mentioned hypotheses, it was assumed that the hydrolysis observed in the case of the solid-supported pipecolic acid **1** was favored by its spatial conformation (different from that of the proline moiety) and was additionally reinforced by the electron-donating effect of an acyl substituent (i.e., cycloalkanecarbonyl). This observation generated a premise that a novel linker based on an N-acylated pipecolic acid handle and cyclohexane-1,4-dicarboxylic acid, used as a spacer between an amino methyl polystyrene resin and pipecolic acid, could be prepared (Scheme 3).

Linker preparation: Pipecolic polystyrene resin **1** (Pip-PS resin) was prepared by using two alternative pathways (Scheme 3). Pathway A consisted of the direct preparation of a linker on a solid support. To this end, cyclohexane-1,4-dicarboxylic acid[21] was coupled to an aminomethyl polystyrene (AM-PS) resin (0.71 mmol g^{-1}). The racemic pipecolic acid methyl ester was then anchored to the solid support **6** through amide coupling. The absence of a free carboxylic acid function was determined by a malachite green



Scheme 2. The unexpected cleavage of pipecolic acid derivatives from the BAL linker.

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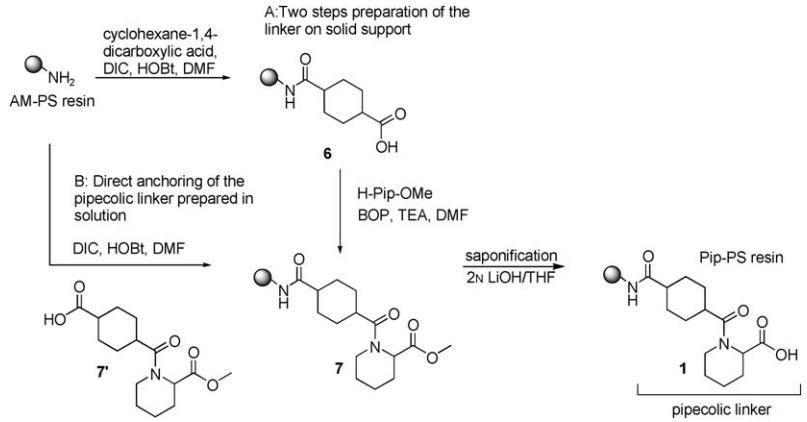

Scheme 3. Preparation of the pipecolic polystyrene (Pip-PS) resin. AM = aminomethyl, DIC = diisopropylcarbodiimide, HOBt = 1-hydroxybenzotriazole, BOP = benzotriazolyl-1-oxytris(dimethylamino)phosphonium hexafluorophosphate, TEA = triethylamine.

test.[22] Alternatively, pipecolic linker methyl ester **7′**[23] was first synthesized in solution and was subsequently introduced by a one-step procedure into an AM-PS resin (pathway B, Scheme 3).

Finally, the methyl ester of resin **7** was hydrolyzed[9] to yield Pip-PS resin **1**. The presence of a new handle attached to the resin was controlled by IR spectroscopy (Figure S1 in the Supporting Information). Loading determination[24] experiments were performed on different Pip-PS resin batches prepared according to pathways A or B, and by diversifying the stereochemistry of pipecolic acid and cyclohexane-1,4-dicarboxylic acid (Table S3 in the Supporting Information). The stereochemistry of the pipecolic linker had a very limited impact on the resin loading. However, the loading of the resin, obtained by using pathway B (0.52 mmol g^{-1}, 86% yield),[25] was slightly enhanced when compared with that obtained by following pathway A (0.49 mmol g^{-1}, 82% yield). The lower loading value determined for the resin, obtained by using pathway A, was probably due to cross-linking occurring during the introduction of cyclohexane-1,4-dicarboxylic acid. This hypothesis was supported by swelling experiments performed on resin samples (3.3 mL g^{-1} for the resin prepared by direct coupling of a linker synthon versus 3.1 mL g^{-1} for the resin prepared by the two-step procedure A).

Amine anchoring: The use of the pipecolic linker was first demonstrated by anchoring amines and alcohols through amide and ester bonds, respectively. Model amines were attached to resin **1**[26] and were then submitted to several structural modifications (Scheme 4). Fmoc-1-amino-3-aminopropane was chosen as a primary amine model, piperazine as a secondary amine model, as well as methyl 2-amino benzoate and N1-Fmoc benzene 1,4 diamine as models of aromatic amines. The amino acid methyl esters of alanine, phenylalanine, and aminoisobutyric acid were also used. The results are presented in Table 1.

Secondary and primary amines, including amino acid methyl esters, were successfully coupled to the support by using BOP activation in the presence of DIEA in DMF, with excellent purities and good yields. In the case of the model primary aromatic amine, methyl 2-amino benzoate, moderate yields were obtained by means of the above-described activation (22% yield). For this reason, we decided to investigate other coupling reagents for aromatic amines. The most efficient method was found when HATU activation was carried out in the presence of TMP in dimethylformamide; the yield being improved up to 98% (a 99% purity). In general, the use of the pipecolic linker significantly improved the resin anchoring of amines compared with trityl linkers. For example, starting from a 2-chlorochlorotrityl PS resin (1.6 mmol g^{-1}),[27] the loading of a primary amine of the amino acid side chain (Orn, Lys) was below 0.3 mmol g^{-1} (<25%). The bulky α-aminoisobutyric acid methyl ester (H-Aib-OMe) was successfully attached to the support under simple BOP activation (**12c**) with a 75% yield, which is a significant improvement when compared with the anchoring of H-Aib-OMe on a 2-chlorochlorotrityl PS resin (yield <12%).

Reverse solid-phase peptide synthesis (SPPS) and *gem*-diamino-derivative synthesis: The Pip linker was also used for SPPS in the reverse N-to-C direction. For this purpose, a Pip linker should be stable during removal of C-terminal protection. To examine this important aspect, a solid-supported bulky Aib methyl ester was subjected to saponification with 2N LiOH in THF, and was then coupled to H-Phe-OMe (Scheme 5).The effectiveness of the coupling reaction was verified by using a malachite green colorimetric test. After TFA treatment for 120 min, the dipeptide H-Aib-Phe-OMe (**23**) was obtained with a good yield and purity.[28] The Pip linker was also used for the solid-phase synthesis of *gem*-diamino derivatives according to a general concept involving the Hoffman rearrangement.

The adoption of this strategy for solid-supported chemistry seems of great importance, since it allows the synthesis of retro-inverso or retro peptides in an easy way, avoiding a tedious preparation of unstable *gem*-diamino derivatives in solution.[9] To this end, H-Leu-NH_2 was coupled to a Pip handle. Subsequent treatment with bistrifluoroacetoxy iodobenzene and pyridine afforded the *gem*-diamino acid derivative of leucine **25**, anchored to a solid support through an amide bond (Scheme 6). The coupling to Fmoc-Phe-OH gave the supported pseudodipeptide **26**. TFA-mediated

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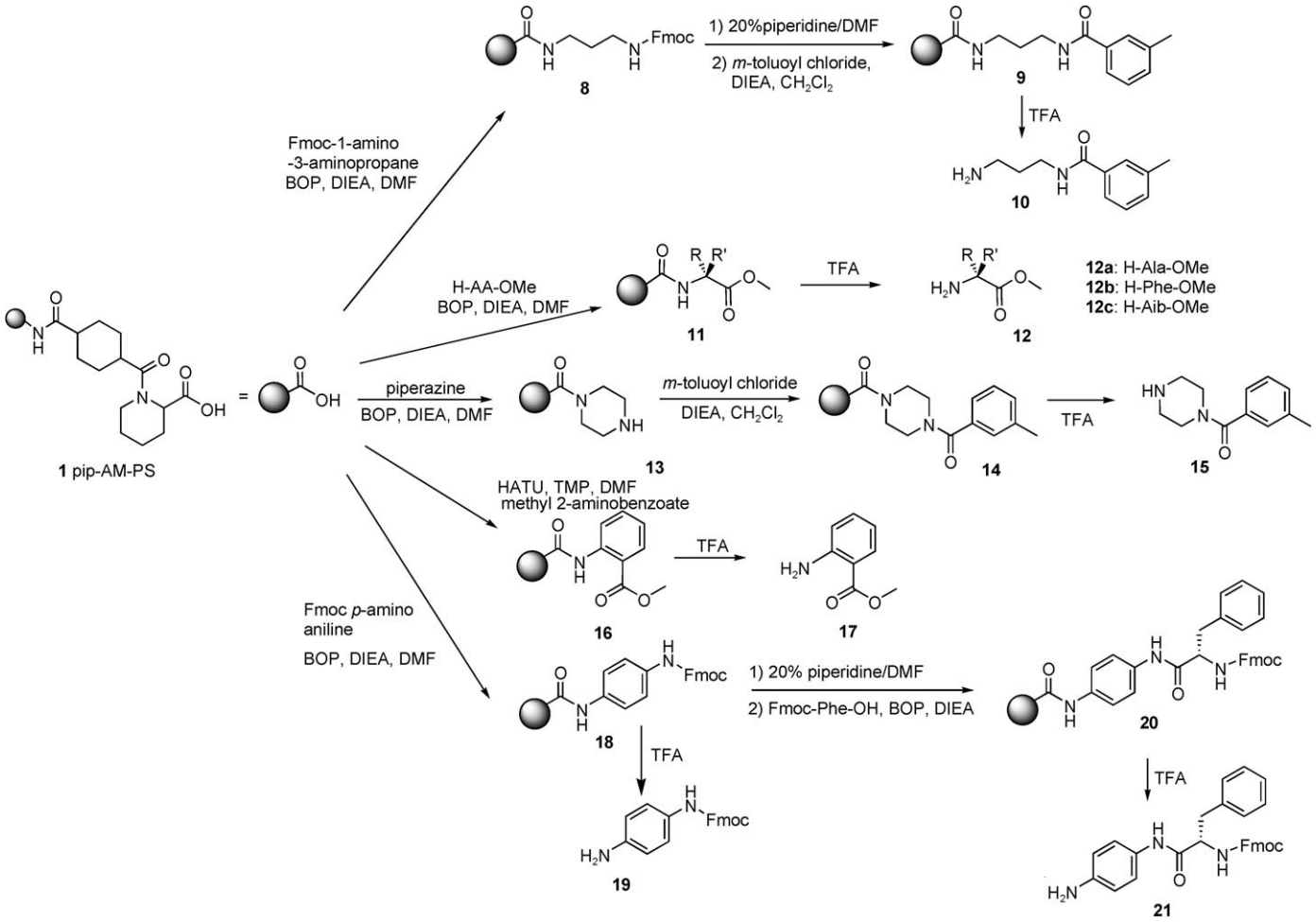
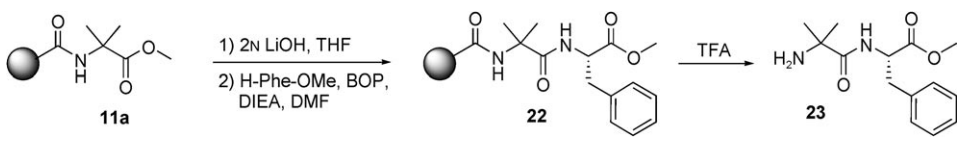


Scheme 4. Amine anchoring on the pipecolic linker. Fmoc = 9-fluorenylmethoxycarbonyl, AA = amino acid, DIEA = ethyldiisopropylamine, HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-b]pyridine-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide, TMP = 2,4,6,-trimethylpyridine.

Table 1. Cleavage results from modifications of model amines attached to resin **1**.

	Purity [%][a]	$[M+H]^+$ found	Yield [%][b]		Purity [%][a]	$[M+H]^+$ found	Yield [%][b]
10	98	193.4	93	**27**	92	472.6	85
12a	99	103.1	82	**29**	98	356.2	44
12b	98	179.1	91	**31**	97	342.0	62
12c	99	117.1	75	**34**	92	412.9	55
15	98	205.1	77	**35**	93	489.6	49
17	97	152.3	93	**37**	98	418.6	54
19	99	331.2	90	**40**	95	489.6	45
21	97	478.2	91	**41**	97	565.6	42
23	95	265.1	92	**44**	92	701.4	34

[a] Purity percentages were calculated by peak area integration during HPLC analysis of cleaved compounds at a sum of wavelengths between 200 to 270 nm [b] Yield were calculated by weighting the cleaved products; cleavage was performed for 120 min under TFA treatment.



Scheme 5. The reverse N-to-C SPPS of H-Aib-Phe-OMe.

cleavage released the pseudo-dipeptide **27**, Fmoc-Phe-g-DLeu-H, in 95% yield and 92% purity.

Alcohol anchoring: Regarding the simple reaction sequence that was performed for the purpose of attaching the amine building blocks to the Pip handle, we proposed that similar reaction conditions could be used for introducing an alcohol derivative through an ester linkage. An amino acid side-chain anchoring strategy may also be of help for the generation of N-to-C cyclic peptides, for the coupling of peptide fragments, or for C-terminus-modified peptides using Fmoc or allyloxycarbonyl (Alloc) chemistry. The primary alcohol function of serine, a secondary alcohol of threonine, and the phenolic function of tyrosine were used, and a cycle of deprotection/coupling steps on the side-chain-grafted amino acid were performed to demonstrate this strategy (Scheme 7).

An ester bond between the Pip handle and the side chains of amino acids was formed by using BOP/DIEA coupling.

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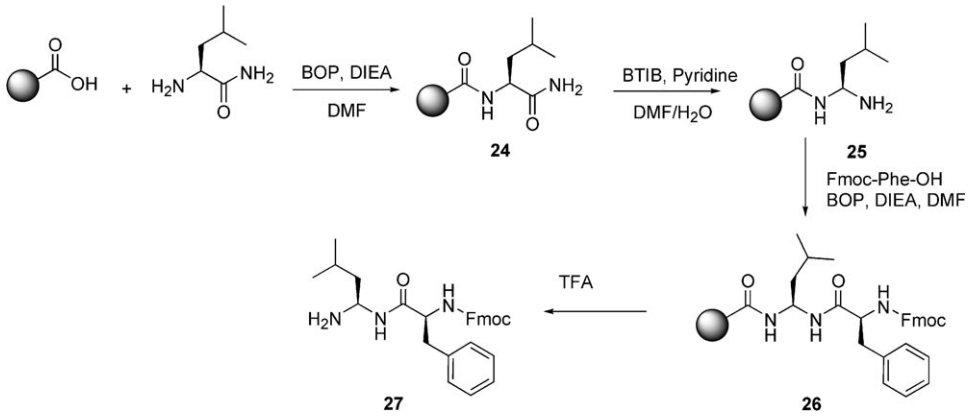
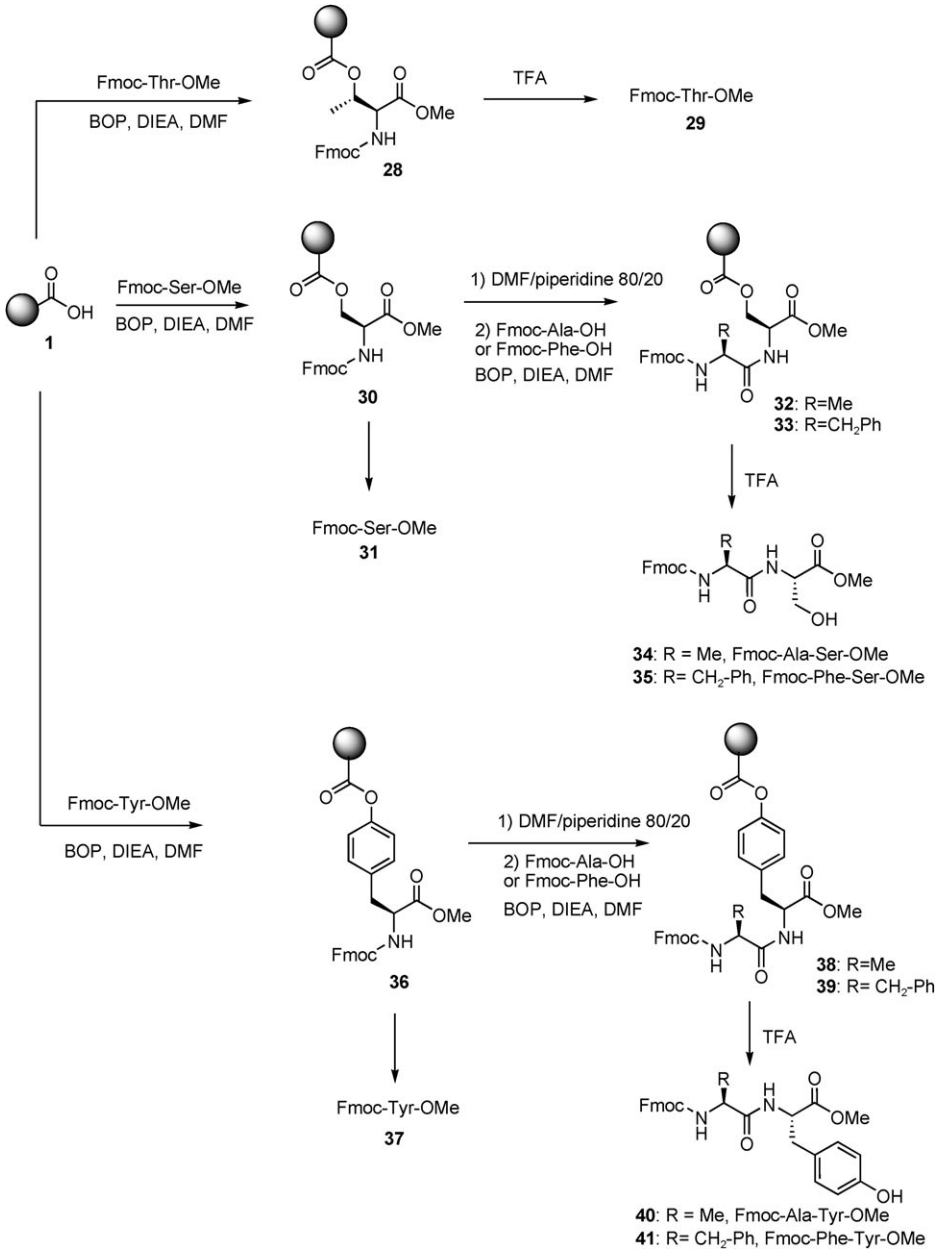


Scheme 6. Synthesis of Fmoc-Phe-g-DLeu-H. BTIB = bis(trifluoroacetoxy)iodobenzene.



Scheme 7. Alcohol anchoring to the pipecolic linker.

Surprisingly, the use of DIC/DMAP or other uronium-mediated activation (e.g., HATU/DIEA) did not yield better results than the BOP/DIEA-mediated esterification. In general, alcohol immobilization was less efficient than amine loading (44, 54, and 62% yields for threonine, tyrosine, and serine, respectively). These results must be analyzed in perspective, since it is difficult to load primary alcohols onto trityl-based resins,[27] the experimental loading yields are below 20% when compared with the theoretical maximum loading. The possibility of continuing Fmoc SPPS on side-chain anchored Fmoc amino acids was also investigated. Resins **30** and **36** were submitted to Fmoc deprotection and were then coupled to either Fmoc-Phe-OH or Fmoc-Ala-OH. Dipeptides **34**, **35**, **40**, and **41** were successfully cleaved from the solid support by TFA treatment, showing moderate yields (42–55%) and, notably, high purities (Table 1).

C-terminal hydrazide peptide synthesis: Finally, the reported Pip-PS resin **1** was coupled to hydrazine by BOP/DIEA activation to yield the supported hydrazide **42** (Scheme 8). The model peptide sequence Ac-His-DPhe-Arg-Trp[29] was synthesized by using standard SPPS methods[30] on hydrazide resin **42** to yield the fully protected supported peptide **43**. The latter peptide was finally treated with a mixture of TFA/TIS/H_2O for 120 min to yield the designed C-terminal hydrazide deprotected peptide **44** with a 92% purity.[31]

Linker stability: To complete our study with the Pip linker, its acid sensitivity was studied on amide resin **8** and on ester resins **30** and **36** by using the following cleavage solutions:

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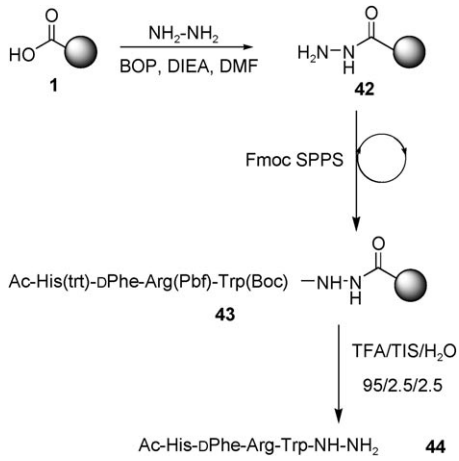

Scheme 8. Preparation of C-terminal hydrazide peptides. TIS = triisopropyl silane.

100% TFA, 10% TFA in CH_2Cl_2, 1% TFA in CH_2Cl_2, and 10% AcOH in CH_2Cl_2. Aliquots of the solution were collected at different times over 24 h and were analyzed by HPLC. Ester and amide linkages behaved almost in the same way under acidic conditions. The cleavage curves of resin **8** are presented in Figure 1.[32]

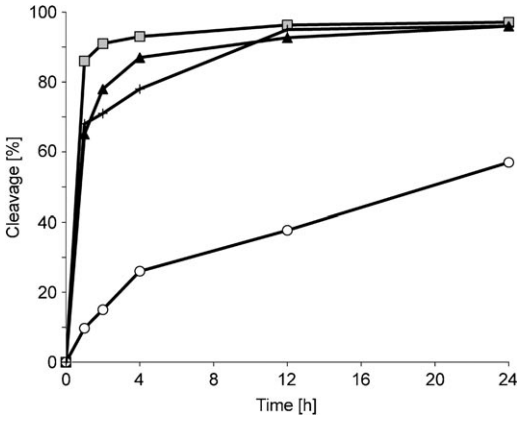

Figure 1. Cleavage of resin **8**. □: 100% TFA, ▲: 10% TFA, ○: 1% TFA, +: 10% AcOH.

In general, the Pip linker was not stable under acidic conditions, that is, after one hour treatment with TFA of the amide and ester linkage, more than 80% of the product was released from the resin, and the product was liberated from the support after 12 h treatment with neat TFA. The linker was not completely stable in 1% TFA either, since after one hour 10% of the product was released from the support.

Conclusion

On the basis of the side reaction observed during arylpiperazine library generation, we developed a new linker for a solid-phase synthesis, based on pipecolic acid. Upon acid treatment, the mechanism involving an oxazolinium-5-one intermediate cleaved an amide or an ester bond and released amines or alcohols. The linker was demonstrated to effectively and simply anchor different functional groups (amines and alcohols). Further examples of the reverse SPPS, a pseudo-peptide synthesis, and amino acid side-chain anchoring shows the versatility of that new linker. Kinetic cleavage studies clearly indicated the acid lability of this linker, which can be used as an alternative to the trityl linker in Fmoc-based synthesis strategies. Regarding the straightforward anchoring, the attachment of other nucleophiles to a solid support through the Pip handle is under investigation and will be reported in due course.

Experimental Section

Preparation of the Pip linker functionalized PS resin 1 (Pathway A, Two-step procedure; Scheme 3)

*Attachment of cyclohexane-1,4-dicarboxylic acid (resin **6**)*: *cis*/*trans*-Cyclohexane-1,4-dicarboxylic acid (*cis*/*trans* 77:23; 5.16 g, 30.0 mmol, 8.45 equiv) and HOBt (4.05 g, 30.0 mmol, 8.45 equiv) were dissolved in DMF (20 mL), then DIC (4,69 mL, 30.0 mmol, 8.45 equiv) were added and the mixture was gently stirred for 10 min. It was then added to aminomethyl-PS resin (5 g, 0.71 mmol g^{-1}, 3.55 mmol) preswollen in CH_2Cl_2, and the reaction mixture was gently shaken for 6 h. After filtration, the resin was washed with DMF (2×), MeOH, and CH_2Cl_2 (2×), and finally dried at room temperature, under vacuum, to yield resin **6**. The coupling efficiency was checked by Kaiser and 2,4,6-trinitrobenzyenesulfonic acid (TNBS) tests.

Attachment of D/L*-pipecolic acid methyl ester (resin **7**)*: D/L-Pipecolic acid methyl ester hydrochloride (**46**; 2.15 g, 12 mmol, 3.4 equiv) was coupled to resin **6** by using BOP (5.30 g, 12 mmol, 3.4 equiv), as activating agents, in the presence of DIEA (5.22 mL, 30 mmol, 8.45 equiv). The reaction mixture was allowed to react for 2 h in DMF, and the process was repeated once more. Finally, the resin was washed with DMF (2×), MeOH, and CH_2Cl_2 (2×), and dried at room temperature under vacuum to yield resin **7**.

*On-resin hydrolysis of pipecolic acid methyl ester (resin **1**)*: A mixture of a 2 M aqueous solution of lithium hydroxide and tetrahydrofuran (200 mL 30:70 v/v) was added to resin **7**. The resin was allowed to agitate on an orbital shaker at room temperature for 24 h. The resin was washed with H_2O (3×), MeOH (2×), and CH_2Cl_2 (3×) to yield Pip-PS resin **1**. The resulting resin was dried under vacuum for 24 h.

Example of amine anchoring on Pip-PS resin: After the Pip-PS resin **1** (200 mg, 98 μmol, 0.49 mmol g^{-1}) was swollen in CH_2Cl_2 for 30 min, and washed with DMF (2×), it was added to DMF coupling solution (5.4 mL) containing BOP (239 mg, 100 mM, 540 μmol, 5.5 equiv), DIEA (140 mg, 188 μL, 200 mM, 1.08 mmol, 11 equiv), and Fmoc-1-amino-3-aminopropane (160 mg, 100 mM, 540 μmol, 5.5 equiv) (Scheme 4). The resin was gently stirred for 2 h and then washed with DMF (2×), MeOH, and CH_2Cl_2 (2×). Then the resin was treated with a mixture of piperidine/DMF (6 mL 20:80 v/v) for 3 min, and subsequently for additional 12 min. After removal of the deprotection solution, the resin was washed by following a standard washing protocol (DMF (2×), MeOH, and CH_2Cl_2 (2×)). In the next stage, the resin was treated with a solution of *m*-toluoyl chloride (39 mg, 100 mM, 540 μmol, 5.5 equiv) and DIEA (140 mg, 188 μL, 200 mM, 1.08 mmol, 11 equiv) in CH_2Cl_2 (5.4 mL). The resin was gently stirred for 2 h and then washed (DMF (2×), MeOH, and CH_2Cl_2 (2×)), and air dried. Cleavage with 100% TFA at room temperature was carried out over 2 h with gentle stirring. The resin was filtered and the TFA solution was evaporated under nitrogen. The residue was dissolved in acetonitrile/water (1:1 v/v) and freeze dried. After lyo-

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philization, compound **10** was obtained as the TFA salt (28 mg, 93%, 98% purity).

Preparation of *gem*-diamino derivative: After the Pip-PS resin **1** (250 mg, 122 μmol, 0.49 mmol g^{-1}), was swollen in CH_2Cl_2 for 30 min, and washed with DMF (2×) it was added to DMF coupling solution (6.75 mL) containing BOP (298 mg, 100 mM, 675 μmol, 5.5 equiv), DIEA (174 mg, 234 μl, 200 mM, 1.35 mmol, 11 equiv), and H-Leu-NH_2 (88 mg, 100 mM, 675 μmol, 5.5 equiv) (Scheme 6). The resin **24** was gently stirred for 2 h and then washed with DMF (3×), MeOH, and CH_2Cl_2 (2×). The resin was then swollen in DMF/H_2O (5 mL 80:20 v/v) containing pyridine (147 μL, 144 mg, 365 mM, 1.82 mmol, 11 equiv) and BTIB (157 mg, 73 mM, 365 μmol, 3 equiv) and was stirred for 1 h. The resin was then washed with DMF (3×) MeOH, and CH_2Cl_2 (2×). The resin **25** obtained was swollen in DMF coupling solution (6.75 mL) containing BOP (298 mg, 100 mM, 675 μmol, 5.5 equiv), DIEA (174 mg, 314 μL, 200 mM, 1.35 mmol, 11 equiv), and Fmoc-Phe-OH (262 mg, 100 mM, 675 μmol, 5.5 equiv). The resin was gently stirred for 2 h and then washed with DMF (3×), MeOH, and CH_2Cl_2 (2×), and air dried. Resin **26** was finally cleaved with TFA over 120 min with gentle stirring. The resin was filtered off and the TFA solution was concentrated under nitrogen. The residue was dissolved in acetonitrile/water (1:1) and freeze dried. After lyophilization, compound **27** was obtained as a TFA salt (60 mg, 85%, 92% purity).

All other data, including alternate resin preparation, loading determination, resin swelling experiments, and synthetic procedures, as well as characterization of the products (LC/MS, NMR spectroscopy analysis), are available in the Supporting Information.

[1] A. F. M. Goodman, L. Moroder, C. Toniolo in *Houben–Weyl, Methods in Organic Chemistry, Synthesis of Peptides and Peptidomimetics, Vol. E22c*, Thieme, Stuttgart, **2002**, pp. 1–847.
[2] A. F. M. Goodman, L. Moroder, C. Toniolo in *Houben–Weyl, Methods in Organic Chemistry, Synthesis of Peptides and Peptidomimetics, Vol. E22d* Thieme, Stuttgart, **2002**, pp. 1–395.
[3] S. Biancalana, D. Hudson, M. F. Songster, S. A. Thompson, *Lett. Pept. Sci.* **2000**, *7*, 291–297.
[4] E. Atherton, C. J. Logan, R. C. Sheppard, *J. Chem. Soc. Perkin Trans. 1* **1981**, 538–546.
[5] F. Albericio, M. Del Fresno, A. Frieden, M. Royo, J. Alsina, K. J. Jensen, S. A. Kates, G. Barany, Peptides: Frontiers of Peptide Science Proceedings of the American Peptide Symposium, 15th, Nashville, **1999**, pp. 37–39.
[6] J. Alsina, K. J. Jensen, F. Albericio, G. Barany, *Chem. Eur. J.* **1999**, *5*, 2787–2795.
[7] J. Alsina, T. S. Yokum, F. Albericio, G. Barany, *J. Org. Chem.* **1999**, *64*, 8761–8769.
[8] K. J. Jensen, J. Alsina, M. F. Songster, J. Vagner, F. Albericio, G. Barany, *J. Am. Chem. Soc.* **1998**, *120*, 5441–5452.
[9] S. Cantel, D. Boeglin, M. Rolland, J. Martinez, J.-A. Fehrentz, *Tetrahedron Lett.* **2003**, *44*, 4797–4799.
[10] S. Cantel, A. Heitz, J. Martinez, J.-A. Fehrentz, *J. Pept. Sci.* **2004**, *10*, 531–534.
[11] A. Hamzé, J. Martinez, J.-F. Hernandez, *J. Org. Chem.* **2004**, *69*, 8394–8402.
[12] G. Subra, M. Amblard, J. Martinez, *Tetrahedron Lett.* **2002**, *43*, 9221–9223.
[13] H. F. Gaertner, K. Rose, R. Cotton, D. Timms, R. Camble, R. E. Offord, *Bioconjugate Chem.* **1992**, *3*, 262–268.
[14] M. B. Pennington, *Lett. Pept. Sci.* **1994**, *1*, 143–148.
[15] P. Zajdel, G. Subra, A. J. Bojarski, B. Duszynska, M. Pawlowski, J. Martinez, *J. Comb. Chem.* **2004**, *6*, 761–767.
[16] C. J. Creighton, T. T. Romoff, J. H. Bu, M. Goodman, *J. Am. Chem. Soc.* **1999**, *121*, 6786–6791.
[17] L. Wei, Y.-Q. Wu, D. E. Wilkinson, Y. Chen, R. Soni, C. Scott, D. T. Ross, H. Guo, P. Howorth, H. Valentine, S. Liang, D. Spicer, M. Fuller, J. Steiner, G. S. Hamilton, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1429–1433.
[18] W. Maison, A. Lutzen, M. Kosten, I. Schlemminger, O. Westerhoff, J. Martens, *J. Chem. Soc. Perkin Trans. 1* **1999**, 3515–3525.
[19] C. Rubini, A. Osler, A. Calderan, A. Guiotto, P. Ruzza, *J. Pept. Sci.* **2008**, *14*, 989–997.
[20] D. Sobolewski, A. Prahl, A. Kwiatkowska, J. Slaninova, B. Lammek, *J. Pept. Sci.* **2009**, *15*, 161–165.
[21] The cheap and commercially available cyclohexane-1,4-dicarboxylic acid [CAS 1076-97-7] was used. It was a mixture of *cis*/*trans* isomers (77:23 determined by NMR spectroscopy analysis). The stereochemistry of cyclohexane dicarboxylic acid was proven to have no influence on the resin loading (see the Supporting Information, Table S3).
[22] M. E. Attardi, G. Porcu, M. Taddei, *Tetrahedron Lett.* **2000**, *41*, 7391–7394.
[23] Preparation of **7′** is described in the Supporting Information.
[24] The loading of resin was verified by UV titration of dibenzofulvene–piperidine adduct released upon piperidine treatment of resin **8**. Calculations for each loading determination are presented in the Supporting Information.
[25] The maximum theoretical loading was calculated by the equation: $0.71/[1+(0.71\times0.265)]=0.60$ mmol g^{-1}. Molecular weight increment from starting AM-PS resin (0.71 mmol g^{-1}) to resin **1**: +0.265 g $mmol^{-1}$.The yields of preparation of the linker were calculated on the basis of the maximum theoretical loading.
[26] The commercially available *cis*/*trans* (77:23) cyclohexyl dicarboxylic mixture and D/L-pipecolic acid were chosen to prepare batches of Pip-PS resin **1** (0.49 mmol g^{-1}) by using convenient two-step solid-phase procedure A for the following experiments of this study.
[27] A. Bernhardt, M. Drewello, M. Schutkowski, *J. Pept. Res.* **1997**, *50*, 143–152.
[28] As far as SPPS in the reverse N-to-C direction is concerned, special attention should be paid to oxazolone-mediated epimerization. In our study, we used a nonepimerisable AIB residue to demonstrate the feasibility of the anchoring strategy and the methyl ester deprotection.
[29] J. R. Holder, R. M. Bauzo, Z. M. Xiang, C. Haskell-Luevano, *J. Med. Chem.* **2002**, *45*, 3073–3081.
[30] M. Amblard, J.-A. Fehrentz, J. Martinez, G. Subra, *Mol. Biotechnol.* **2006**, *33*, 239–254.
[31] 4% of hydrazone formed with acetone was detected by LC/MS.
[32] Other curves are presented in the Supporting Information.

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