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A Nonacid Degradable Linker for Solid-Phase Synthesis

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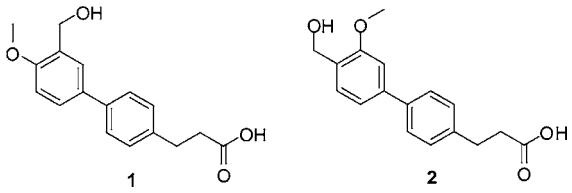
ABSTRACT



Synthesis and applications of two new nonacid degradable linkers as an alternative to the Wang linker for solid-phase synthesis are described. Resin from linker 2 looks superior to linker 1 in terms of yields for both anchoring of the first building block and cleavage and in terms of higher purity of the final product. Use of linker 2 avoids side reactions associated with the use of Wang resin due to an undesired cleavage during final acid treatment.

Solid-phase synthesis is a convenient and suitable method for the synthesis of peptides and small molecules.[1] A key feature of the solid phase approach is the linker,[2] and therefore, recent years have witnessed an explosion in the research and development of novel linkers for polymer-supported synthesis[3] as well as in the publication of several reviews[4] covering this area. Originally, solid-phase synthesis focused on the synthesis of peptides and nucleotides, but the advent of combinatorial chemistry techniques triggered the demand for a wider range of linkers. The ideal linker should allow easy attachment of the starting material to the support, be stable under a broad variety of reaction conditions, and enable selective cleavage at the end of a synthesis without causing damage to the product or cleavage of the proper linker, which could be reincorporated into the final product. This is more important in the case of a nonintegral linker that is attached to the resin core through a chemical bond.[2]

In our group, different acid-labile linkers were used for the synthesis of small organic molecule libraries,[5] and in some cases, unexpected results due to the cleavage of the linker during the synthesis[6] or in the cleavage stage[7] were observed. The side product formed can contaminate the crude

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(1) (a) *Solid Phase Synthesis: A practical guide*; Albericio, F., Kates, S. A., Eds.; Marcel Decker: New York, 2000. (b) *Handbook of Combinatorial Chemistry*; Nicolau, K. C.; Hanko, R.; Hatwig, W., Eds.; Wiley-VCH: Winheim, 2002.

(2) Linkers, as defined by Bradley et al. (see ref 4d), can be considered simply as immobilized protecting groups and will be classified into one of two types: (i) Integral linkers in which part of the solid support core forms part or all of the linker and (ii) nonintegral (or grafted) linkers in which the linker is attached to the resin core. A linker which has been prepared in solution will be defined as a unloaded linker.

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product, and even more damaging, back alkylation of the target compound can occur.

This side reaction is more severe when strong acid conditions are used to cleave the final product.[8] However, undesired cleavage can take place with a Wang-type resin, which requires milder TFA conditions for liberating the final product (Figure 1). Thus, in peptide synthesis, Tsikaris et

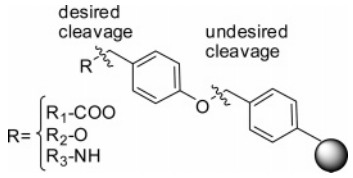
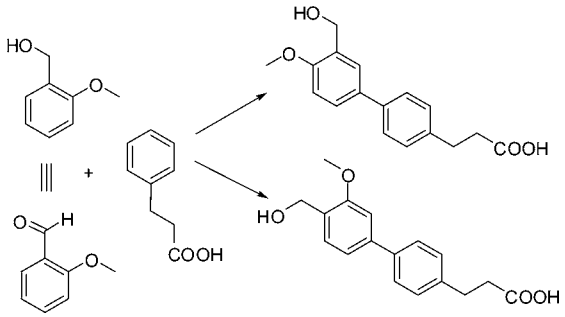


Figure 1. Dual cleavage of Wang-type resin.

al.[9] have described the incorporation of the *p*-hydroxybenzyl moiety cleaved from the Wang resin into the N of the C-terminal amide of a peptide during TFA cleavage. Similarly, Martinez et al.[10] have described the alkylation of the indol ring of Trp-containing peptides by the *p*-hydroxybenzyl moiety. Furthermore, Stanger and Krchnak have reported formation of *O*-(4-hydroxy)benzyl derivatives.[11] The use of the Wang resin for the solid-phase preparation of small molecules has led to the introduction of impurities due to the undesired cleavage from the resin (no cleavage at the benzyl position) or from a back alkylation of the *p*-hydroxybenzyl cation in the case of furopyridine and furoquinoline target derivatives.[12]

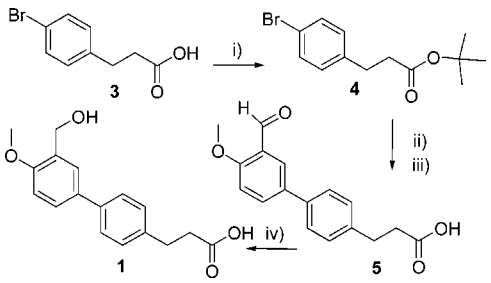
The goal was to develop a more stable Wang-type linker able to liberate the compound with 20−95% TFA in DCM. As the Wang linker is based on a *p*-alkoxybenzyl alcohol, the new linker should contain a benzyl alcohol activated by a non cleavable electron-donating group in either the ortho or para position and then a carboxylic group for easy attachment to an amino resin.[13] Furthermore, the linker should be easy to prepare. The simplest activated benzyl alcohol is the 2-methoxybenzyl alcohol,[14] which can be masked by the use of the corresponding aldehyde. The carboxylic acid can be attached to another phenyl ring, and both rings can be bound through a Suzuki coupling reaction. Thus, these two moieties can be coupled through two different positions to give two regioisomer linkers (Scheme 1).

Scheme 1. Possible Structures for the Linkers Proposed



First of all, linker **1** was synthesized from the commercially available 3-formyl-4-methoxyphenylboronic acid and the 3-(4-bromophenyl) propanoic acid, which was first protected in the form of the *tert*-butyl ester, through a Suzuki reaction. Protection of the carboxylic acid is translated into a better yield for the coupling reaction. Finally, the acidolysis of the *t*-butyl ester followed by the reduction of the aldehyde render the target linker **1** with an overall yield of 64% (Scheme 2).

Scheme 2. Synthesis of Linker **1**[a]



[a] i) Isobutylene, DCM, −78 °C; ii) $Pd(PPh_3)_4$, toluene−EtOH (9:1), 3-formyl-4-methoxyphenylboronic acid, 24 h, 90 °C; iii) DCM−TFA (1:1); iv) $NaBH_4$.

Synthesis of linker **2** was performed in the same way as linker **1** (Scheme 3), but because the boronic acid required is not commercially available, it was synthesized from 2-methoxy-4-bromobenzaldehyde (**6**). Linker **2** was prepared with an overall yield of 80%.

Linkers **1** and **2** were loaded onto aminomethyl-polystyrene resin with HBTU and DIEA [until no primary amine

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(8) For instance, Gu and Silverman (see ref 3b) designed a backbone linker stable to reflux TFA.

(9) Stathopoulos, P.; Papas, S.; Tsikaris, V. *J. Pept. Sci*. **2006**, *16*, 227−232.

(10) Giraud, M.; Cavalier, F.; Martinez, J. *J. Pept. Sci.* **1999**, *5*, 457−461.

(11) Stanger, K. J.; Krchnack, V. *J. Comput. Chem.* **2006**, *8*, 652−654.

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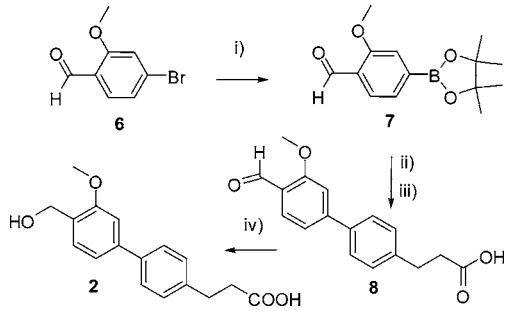
(13) Gu and Silverman (ref 3b) incorporated the precursor of their backbone linker to the resin through a metal-catalyzed coupling reaction.

(14) Phenylmethylethers are rather stable to acid conditions, and even in the case of cleavage, the methyl cation formed is less reactive in comparison with benzyl cations.

Scheme 3. Synthesis of Linker **2**[a]



[a] i) bis(pinacolato) diboron, $PdCl_2$(dppf), dioxane, 24 h, 90 °C; ii) **4**, $Pd(PPh_3)_4$, toluene−EtOH (9:1), 24 h, 90 °C; iii) DCM−TFA (1:1); iv) $NaBH_4$.

was detected by the Kaiser test[15] (12 h)], obtaining resin **9** from linker **1** and resin **10** from linker **2**. Resins were analyzed by HRMAS observing the total disappearance of the proton of the CH_2-NH_2 from the aminomethyl resin and the appearance of the proton corresponding to CH_2OH and OCH_3, confirming the colorimetric test. Once the resins were characterized, the stability of the linkers in acid conditions was evaluated using 50% or 95% TFA at 60 °C during 1 h by analyzing the filtrates by HPLC and HPLC-MS. No cleavage of the linker was observed in any of the cases, but a small percentage of linker (**1** or **2**) was observed in all four along with HOBt coming for the anchoring to the resin. For this reason, extensive washings of the resin were done.

Once the stability of the linkers anchored to the resin was validated, the scope and limitations of these linkers were tested. Thus, Fmoc-Leu-OH was incorporated onto resins **9** and **10** by several methods for 4 h, and the yield of the piperidine-dibenzofulvene adduct was calculated by means of UV (Table 1).[16]

Table 1. Coupling Conditions Tested with Both New Resins

entry	resin	coupling conditions	treatments	% coupling
1	**9**	DIC/Fmoc-Leu-OH/HOBt/DMAP	1 × 1 h	66
		(5:5:5:0.03)	2 × 1 h	100
2		DIC/Fmoc-Leu-OH/DMAP	1 × 1 h	96
		(3:6:0.03)	2 × 1 h	100
3		HBTU/Fmoc-Leu-OH/DIEA[a]	1 × 1 h	90
		(5:5:10)	2 × 1 h	100
4	**10**	DIC/Fmoc-Leu-OH/HOBt/DMAP	1 × 1 h	17
		(5:5:5:0.03)	2 × 1 h	23
5		DIC/Fmoc-Leu-OH/DMAP	1 × 1 h	73
		(3:6:0.03)	2 × 1 h	100
6		HBTU/Fmoc-Leu-OH/DIEA[a]	1 × 1 h	75
		(5:5:10)	2 × 1 h	98

[a] DMAP was not used.

Table 1 shows that the symmetrical anhydride is the best method for both linkers (entries 2 and 5) and that linker **9** is acylated more easily than linker **10**.

Cleavage of the anchored Fmoc-Leu-OH to resins **9** and **10** was studied with different TFA concentrations at room temperature and carrying out one or two treatments. As shown in Table 2, resin **10** is more labile than **9**. Thus, 25%

Table 2. Yields and Purities of Cleaved Products

entry	resin	% TFA	time	% yield	% purity[a]
1	**9**	95	1 h	81	83
2		95	2 × 1 h	95	85
3		50	1 h	53	87
4		50	2 × 1 h	70	85
5	**10**	95	1 h	98	98
6		95	2 × 1 h	98	100
7		50	1 h	92	99
8		50	2 × 1 h	95	100
9		25	1 h	97	97
10		25	2 × 1 h	95	100

[a] Purity measured by HPLC.

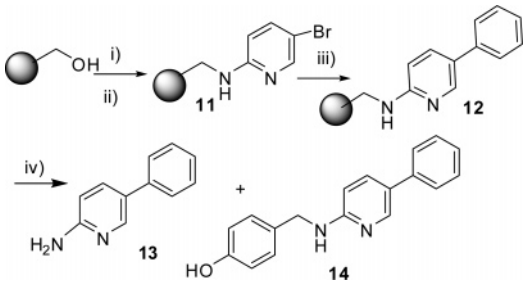
TFA is enough for the total cleavage of the amino acid (#10) instead of 95%, which is required for resin **9** (#2). Furthermore, a greater purity of Fmoc-Leu-OH is observed in the cleaved samples performed on resin **10** (97−100% purity for resin **10** vs 83−87% purity for resin **9**).

These results are in line with those expected because in resin **10** the electron-donating phenyl group is at the para position with respect to the hydroxymethyl, increasing the stability of the benzylic carbocation.

Next, to compare the stability of resins **9** and **10** to commercial Wang resin, solid-phase synthesis of 2-aminopyridine derivatives as well as of several model peptides was performed.

For the synthesis of 2-aminopyridine derivatives (Scheme 4),[17] the monitoring of the bromination and the loading of

Scheme 4. Synthesis of the 2-Amino-5-phenylpyridine[17]



[a] i) PBr_3, DCM, 16 h; ii) 2-amino-5-bromopyridine, CsI, proton sponge, 24 h, 80 °C; iii) phenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, DMF, 24 h, 90 °C; iv) DCM−TFA.

the 5-bromopyridin-2-amine was carried out by HRMAS to confirm the total conversion in each step. The cleavage of

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(17) Zhu, S.; Shi, S.; Gerritz, S. W.; Sofia, M. J. *J. Comb. Chem.* **2003**, *5*, 205−207.

the three resins was performed by two treatments with different TFA solutions for 1 h each, at different temperatures (Table 3).

Table 3. Cleavage after the Synthesis of the 2-Amino-5-phenylpyridine

no.	resin	cleavage solution	temp	yield (%)	**13** (%)[a]	**14** (%)[a]
1	Wang	TFA−DCM (95:5)	rt	100	40	60
2	Wang	TFA−DCM (1:1)	rt	76	45	55
3	**9**	TFA−DCM (95:5)	rt	15	97	–
4	**9**	TFA−DCM (95:5)	60 °C	60	96	–
5	**10**	TFA−DCM (95:5)	rt	22	98	–
6	**10**	TFA−DCM (95:5)	60 °C	100	95	–

[a] Purity measured by HPLC at 210 nm.

As shown in Table 3, no byproducts were detected with the two linkers (#3−6), and the target compounds were obtained with good purity. On the other hand, Wang resin released the unwanted compound **14** through an undesirable cleavage by the benzylic ether (#1−2). Furthermore, Wang resin showed a better lability allowing a total cleavage of the compound at room temperature. This could be related to the dual cleavage point. Resin **10** was optimal because total cleavage was obtained at 60 °C.

A model peptide Leu-enkephalin (H−Tyr-Gly-Gly-Phe-Leu−OH) was synthesized on the two resins (**9** and **10)** using the Fmoc/^{t}Bu strategy. Final cleavage was performed with TFA−TIS−H_2O (95:2.5:2.5) (2 × 1 h). Table 4 shows that resin **10** (#3−4) renders better purities and yields. Resin **9** (#1−2) resulted in a impurity of the Leu deletion peptide.

Finally, model peptides, H−Tyr-Leu-Ser-Gly-Ala-Asn-Leu-Asn-Leu−OH (**15**), H−Arg-Pro-Gly-Leu-Leu-Asp-Leu-Lys−OH (**16**), and H−Val-Gln-Gly-Glu-Glu-Ser-Asn-Asp-Lys−OH (**17**), were synthesized with excellent purity and yields on resin **10** (Table 5).

In summary, two non acid degradable linkers were synthesized and tested for the solid phase of both peptides and small molecules. Although both linkers form the same carbocation during the cleavage stage, the different position of the phenyl moiety confers different stabilities of this carbocation to both. In this sense, the resin derived from linker **2** gives better results in several parameters: (i) yield of anchoring of the first protected amino acid; (ii) cleavage yield of the final product; and (iii) higher purity of the final product. Any of them render undesired cleavage, which can jeopardize the synthesis. This strategy can be used for developing new acid-labile linkers.

Table 4. Coupling Conditions for the Synthesis of Leu-enkephalin[a]

no.	resin	coupling conditions for the Fmoc-Leu-OH	coupling conditions for the other aa	% purity	% mass recovered
1	**9**	2×(DIC/AA/DMAP) (5:10:1)	HBTU/DIEA/AA (5:10:5)	62	43
2		2×(HBTU/DIEA/AA)[b] (5:10:5)	HBTU/DIEA/AA (5:10:5)	63	48
3	**10**	2×(DIC/AA/DMAP) (5:10:1)	DIC/AA/HOBt (5:10:1)	93	98
4		2×(HBTU/DIEA/AA)[b] (5:10:5)	HBTU/DIEA/AA (5:10:5)	92	99

[a] Purity measured by HPLC at 254 nm. [b] DMAP was not used.

Table 5. Results of the Synthesis of Peptides **15**−**17**

no.	peptide	yield (%)	purity (%)[a]
1	**15**	93	97
2	**16**	88	98
3	**17**	97	92

[a] Purity measured by HPLC at 254 nm.

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Supporting Information Available: Synthesis and NMR spectroscopic data of the compounds and the new resins, protocols used for peptide synthesis, and details of HPLC measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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