Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 45 (2004) 5237–5241
Tetrahedron Letters

Dual linker with a reference cleavage site for information rich analysis of polymer-supported transformations

Viktor Krchňák[a,*] and Greg A. Slough[b]

[a]*Department of Chemistry and Biochemistry, 251 Nieuwland Science Center, University of Notre Dame, Notre Dame, IN 46556, USA*
[b]*Chemistry Department, Kalamazoo College, 1200 Academy St., Kalamazoo, MI 49006, USA*

Received 3 March 2004; revised 30 April 2004; accepted 7 May 2004

Abstract—Two dual linker systems with specific reference cleavage sites were designed and synthesized to accelerate and simplify development and optimization of reaction conditions for solid-phase synthesis. The dual linker allows simple evaluation of cleavage rate of polymer-supported compounds from the linker and, at the same time, ensures that all resin-bound components are cleaved from the solid support. The dual linker **4** was assembled from two Wang linkers connected by a three carbon spacer. The linker **9** was synthesized using the PAL and HMPB linkers.

Optimization of reaction conditions is a prerequisite for successful solid-phase synthesis. Whereas the critical task of solution phase combinatorial synthesis is the isolation of the product, the most time-consuming step of solid-phase combinatorial synthesis is optimization of reaction conditions; the product isolation cannot be simpler. The final step of a chemical transformation on solid phase includes cleavage of the resin-bound reaction components followed by analysis of the cleaved sample. To obtain meaningful information regarding the composition of components on solid phase, all components need to be cleaved from the support (a textbook example of a reaction, where the starting synthon is not cleaved is acylation of polymer-supported *N*-alkylated benzylamines). Information that cannot be obtained from this simple experiment is the rate of cleavage from the solid support. An independent quantification analysis is required in order to assess the cleavage yield.

We report a general, yet very simple, concept that yields cleavage data for all polymer-supported components and, at the same time, assays the cleavage rate. This is possible by carrying out the synthesis on a polymer-supported dual linker with a reference cleavage site consisting of two sequential linkers separated by a spacer (Fig. 1). The spacer is designed to allow its cleavage from the Linker 1 under standard conditions. Linkers 1 and 2 may be, but do not need to be, the same linkers. However, both linkers need to be cleavable under the same reaction conditions. Simultaneous cleavage of compounds from both linkers by the same reagent gives cleavage of all polymer-supported components and allows one to fine tune the cleavage conditions for complete removal of the synthetic product. Thus, the presence of the desired compound still attached to Linker 2 and the spacer indicates incomplete cleavage from Linker 2. Alternatively, observation of the desired compound free from Linker 2 indicates complete cleavage.

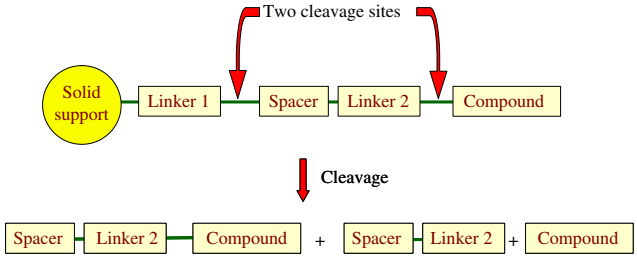


Figure 1. The concept of two dual linkers with a reference cleavage site.

The concept of dual linkers is not a new one and was recently reviewed.[1] In 1979 Merrifield described a

Keywords: Solid-phase synthesis; Dual linker; Cleavage rate.
* Corresponding author. Tel.: +1-574-6315113; fax: +1-574-6316652; e-mail: vkrchnak@nd.edu

0040-4039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2004.05.029

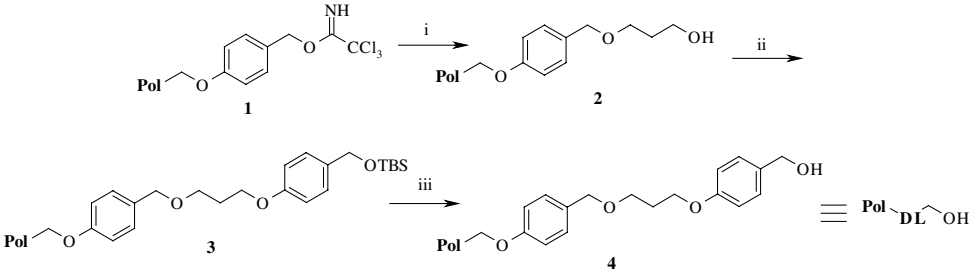
multi-detachable resin consisting of two linkers with different cleavage characteristics, which allows cleavage of either free or protected peptides.[2,3] Geysen used the dual linker concept to incorporate a coding moiety between two linkers that facilitated library encoding.[4] Two different linkers with orthogonal cleavage characteristics have been connected via an analytical enhancer, that facilitated UV and MS detection of the cleaved product by incorporating a strong UV chromophore[5,6] and MS sensitizer (with an isotope label),[5,7–9] respectively. Current limitations of dual linkers are twofold: (i) the need to synthesize the analytical construct, and (ii) incorporation of functionalities not compatible with all reagents and chemistries used in the synthesis.[1]

In order to prove the concept of the dual linker with a reference cleavage site, we built a tandem from two Wang linkers (Scheme 1). The Wang linker[10] is frequently used in solid-phase synthesis and it allows preparation of a range of compound classes including acids,[10,11] alcohols,[12] and phenols.[12–14] For the preparation of amides[15–19] and amines,[20] one or two additional methoxy groups have been introduced onto the aromatic ring in order to increase the acid mediated cleavage. We connected two Wang linkers via a three methylene carbon spacer in order to install the reference cleavage site; thus quantitative cleavage between the Wang Linker 1 and the spacer is achieved under standard conditions (10% trifluoroacetic acid (TFA) in dichloromethane (DCM) for 30 min).[12]

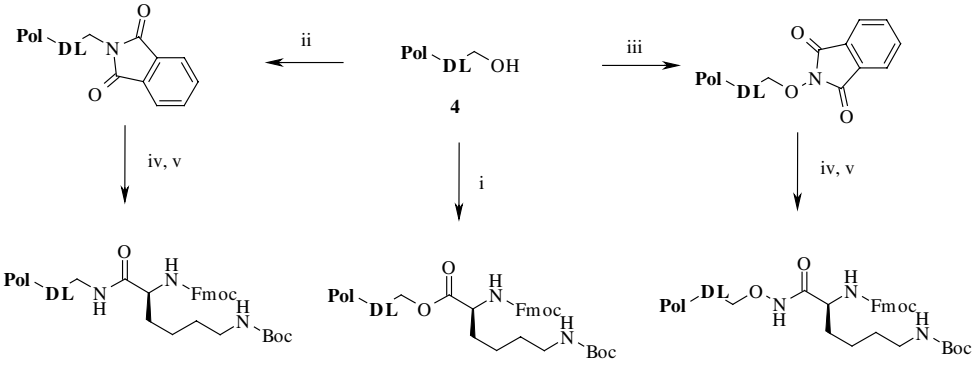
The synthesis of the dual Wang linker is straightforward. Trichloroacetimidate Wang polystyrene—1% divinylbenzene (PS/DVB) resin **1** (NovaBiochem, 0.66 meq/g) reacts with 1,3-propanediol according to the reported procedure.[12] A sample of the resulting resin-bound alcohol **2** was acylated with Fmoc-Ser(Bzl)-OH and the product treated with TFA/DCM. The expected serine derivative Fmoc-Ser-O-$(CH_2)_3$-OH was cleaved in 74% yield with respect to the declared resin substitution level (quantification from HPLC traces at 300 nm). The resin **2** was then coupled to TBS protected 4-hydroxybenzyl alcohol[21] under Mitsunobu conditions[22] to yield the TBS protected dual linker resin **3**.[23] A sample of the resin **3** was acylated with Fmoc-Ser(Bzl)-OH in order to detect any unreacted alcohol and the product was cleaved with 50% TFA in DCM for 30 min. Only traces (<1%) of Fmoc-Ser-$(CH_2)_3$-OH were detected.

Finally, the TBS group was cleaved by TBAF and a sample of the polymer-supported dual linker **4** was exposed to 10% TFA in the presence of 5% Et_3SiH. The expected 3-(4-methylphenoxy)propan-1-ol was detected. Without the use of the scavenger silane, polymerization of the benzyl alcohol derivative in TFA takes place and no simple product can be detected on HPLC traces.

Several model compounds were prepared (Scheme 2) and cleaved with TFA in order to prove the concept of the dual linker with a reference cleavage site. Esterification by Fmoc-Lys(Boc)-OH was carried out by in situ prepared HOBt esters with DMAP catalysis. Fmoc-



Scheme 1. Synthesis of the dual Wang linker. Reagents: (i) 1,3-propanediol, BF_3 (etherate), anhydrous THF, 30 min; (ii) p-HO-Ph-CH_2-OTBS, DEAD, PPh_3, anhydrous THF, 20 °C, overnight; (iii) 0.5 M TBAF, THF, 20 °C, 30 min.



Scheme 2. Synthesis of model compounds. Reagents: (i) Fmoc-Lys(Boc)-OH, DIC, HOBt, DMAP, DMF/DCM, 20 °C, 16 h; (ii) phthalimide, PPh_3, DEAD, anhydrous THF, 20 °C, 16 h; (iii) *N*-hydroxyphthalimide, PPh_3, DEAD, anhydrous THF, 20 °C, 16 h; (iv) hydrazine hydrate, THF/MeOH (1:1), 20 °C, 16 h; (v) Fmoc-Lys(Boc)-OH, DIC, DMF/DCM, 20 °C, 16 h.

Table 1. Relative amount of products **7** and **8** as a function of TFA concentration and time

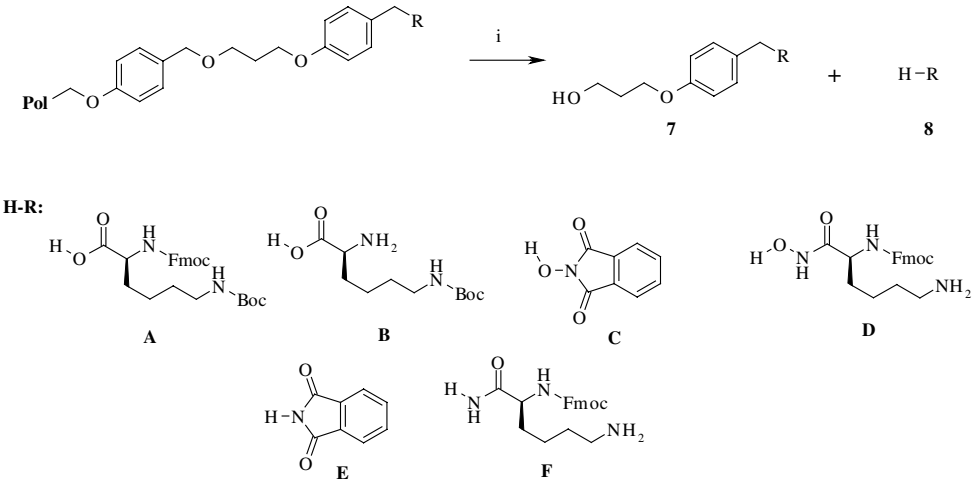
Entry	R	Bond cleaved	TFA/DCM (%)	Time (min)	Product **7** (%)	Product **8** (%)
1	A	C–O	10[a]	30	11	89
2	A	C–O	10[a,b]	30	11	89
3	A	C–O	50	30	<1	>99
4	B	C–O	10	30	82	18
5	C	C–O	1[c]	60	74	26
6	C	C–O	10	30	63	37
7	C	C–O	10	60	<1	>99
8	C	C–O	50	30	<1	>99
9	D	C–O	10	30	71	29
10	D	C–O	50	30	20	80
11	E	C–N	95[b,d]	60	93	7
12	F	C–N	95[b,d]	60	>99	<1

[a] Partial cleavage of the Boc group.
[b] Contains 5% Et_3SiH.
[c] Incomplete cleavage from the Wang Linker 1 (the 'Linker 1—spacer' bond).
[d] TFA ester of **7E** was formed during cleavage, which hydrolyzed in MeOH/water solution.

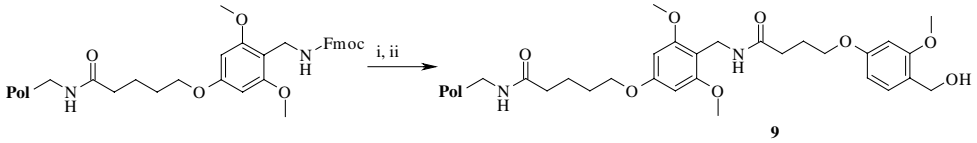
Lys(Boc)-OH was selected as the acylation species since it provides easy quantification using the Fmoc group and the free amino group (after TFA cleavage from the resin) enhances identification by mass spectroscopy. Phthalimide was used to convert the Linker 2 into the amino-functionalized resin.[24] Analogously, *N*-hydroxyphthalimide was used to install the hydroxylamine for the synthesis of hydroxamic acids.[25,26] In both cases, the phthalimide was cleaved with hydrazine and the amino groups were acylated with Fmoc-Lys(Boc)-OH.

The results of cleavage experiments are summarized in Table 1. Trifluoroacetic acid cleavage leads to the formation of two products, **7** and **8** (Scheme 3). The presence of compound **7** indicates incomplete cleavage from the Linker 2. Ester cleavage of N^{α}-Fmoc protected amino acid (entries 1–3) is fast and after 30 min in 10% TFA/DCM only 11% of compound **7A** remains to be cleaved. The presence of the scavenger Et_3SiH does not affect the cleavage rate, however, 3-(4-methylphenoxy)propan-1-ol was detected. In contrast, cleavage of *N*-unprotected amino acid (after Fmoc group cleavage with piperidine in DMF) is substantially slower (only 13% of **8B** cleaved), due to the protonation of the N^{α}-amino group (entry 4). Acylation with Boc-Lys(Fmoc)-OH and subsequent cleavage with TFA showed 84% cleavage, comparable with sample **A**, which was acylated by Fmoc-Lys(Boc)-OH, suggesting that the Boc cleavage occurred only after the product was cleaved from the resin.

Immobilized *N*-hydroxyphthalimide, an intermediate for the synthesis of hydroxamic acids, was more stable when compared to esters (entries 5–8). Quantitative cleavage of *N*-hydroxyphthalimide **8C** from the Wang Linker 2 required 30 min treatment with 50% TFA. Cleavage of hydroxamic acids was substantially slower, 71% of compound **7D** remained intact after 30 min in 10% TFA. Cleavage with 50% TFA showed 20% of the product **7D** (entries 9 and 10). Quantitative cleavage of hydroxamic acid required 95% TFA for one hour. However, under these conditions the Wang linker was partially cleaved from PS/DVB support. Thus, the more acid labile 4-(4-hydroxymethyl-3-methoxyphenoxy)-butyric acid (HMPB) linker[27] is more suitable for the synthesis of hydroxamic acids.



Scheme 3. Cleavage of model compounds. Reagents: (i) TFA in DCM, for concentration and reaction time see Table 1.



9

Scheme 4. Synthesis of the dual amide linker. Reagents: (i) 40% piperidine, DMF, 20 °C, 20 min; (ii) HMPB linker, DIC, HOBt, DMF/DCM, 20 °C, 16 h.

Expectedly, almost no cleavage of the C–N amide bond was observed in any experiment (entries 11 and 12). One hour in 95% TFA (5% water or Et_3SiH) released 7% of the product **8E** from the Linker 2. After acylation with Fmoc-Lys(Boc), compound **8F** was not detected (no corresponding ion current during LC/MS analysis). Thus, cleavage of amides requires a more acid labile linker.[15–19]

The next dual linker was built from linkers designed for the synthesis of nitrogen-containing compounds (cleavage of the C^{α} (benzyl)-N(amide) bond). The linker **9** was assembled from two different linkers, which are both cleavable with reagents such as TFA (Scheme 4). Aminomethylated polystyrene—1% divinylbenzene resin (Advanced ChemTech, 1.2 meq/g) was acylated with the 5-(4-(9-fluorenylmethyloxycarbonyl)aminomethyl-3,5-dimethoxyphenoxy)valeric acid (PAL) linker.[28] The substitution level was evaluated by quantification of Fmoc-NH_2, cleaved from the resin by 10% TFA in 60 min and was found to be 89% with respect to the declared substitution of aminomethylated resin (repeated cleavage did not provide additional product). The cleavage time was estimated by treatment of the PAL linker (not attached to the resin) with TFA. After exposure to 10% TFA for 30 min, 5.8% of intact PAL linker was detected by HPLC. The cleavage of Fmoc-NH_2 was complete after 60 min.

After cleavage of the Fmoc group, the resin-bound amino group was acylated with the HMPB linker.[27] A sample of the resin was reacted with Fmoc-ONSu and treated with TFA. No Fmoc-NH_2 was detected indicating complete coupling of the HMPB linker. The dual linker **9** was reacted with phthalimide and *N*-hydroxyphthalimide under Mitsunobu conditions and the products cleaved when treated with 10% TFA for 30 min (in addition to expected products, a side-product having a strong MS signal at 484 Da was detected). In the case of phthalimide, cleavage from the Linker 2 was incomplete with approximately 46% of phthalimide remaining attached to the HMPB linker. Complete cleavage of phthalimide from the Linker 2 was observed at higher concentration of TFA (50% in DCM) in 30 min. *N*-hydroxyphthalimide was cleaved completely with 10% TFA in 30 min. Complete cleavage was also observed after deprotection of the phthalyl group and acylation by carboxylic acids, indicating that this is the linker of choice for the synthesis of hydroxamic acids.

In summary, the new dual linker with a reference cleavage site can serve in development of solid-phase synthesis. (i) The dual linker ensures cleavage of all resin-bound components, thus allowing identification of all components present on the solid-phase support. (ii) It allows simple estimation of the cleavage rate of polymer-supported compounds from LC/MS traces. (iii) It is suitable for straightforward optimization of cleavage protocol to arrive at the mildest conditions required for quantitative cleavage of particular products from a resin-bound linker, including evaluation of new cleavage cocktails. (iv) The second linker can be replaced by a linker with modified cleavage characteristics (more stable, more labile) in order to arrive at the most suitable linker for given compound classes. (v) The dual linker has a potential to be used for design of new linkers with altered sensitivity towards cleavage conditions. (vi) Finally, the dual linker can assist in determining the reason of low yield. Such poor yields can be caused not only by incomplete cleavage but also by premature loss during synthesis or incomplete extraction of cleaved compound from the solid support. Elimination of incomplete cleavage narrows the determination of the cause.

The synthesis of the dual linker with a reference cleavage site is very simple and straightforward. The dual linker does not incorporate any additional functional group, hence it does not restrict the choice of chemical transformations. This letter described the concept of the dual linker with a reference cleavage site and its application to selection of the optimal linker for the synthesis of hydroxamic acids.

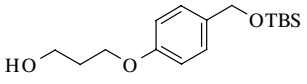
Acknowledgements

The work was supported by the Department of Chemistry and Biochemistry University of Notre Dame.

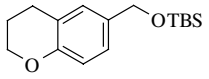
References and notes

1. Congreve, M. S.; Ley, S. V.; Scicinski, J. *Chem. Eur. J.* **2002**, *8*, 1769–1776.
2. Tam, J. P.; Tjoeng, F. S.; Merrifield, R. B. *Tetrahedron Lett.* **1979**, *20*, 4935–4938.
3. Tam, J. P.; Tjoeng, F. S.; Merrifield, R. B. *J. Am. Chem. Soc.* **1980**, *102*, 6117–6127.
4. Geysen, H. M.; Wagner, C. D.; Bodnar, W. M.; Markworth, C. J.; Parke, G. J.; Schoenen, F. J.; Wagner, D. S.; Kinder, D. S. *Chem. Biol.* **1996**, *3*, 679–688.
5. Williams, G. M.; Carr, R. A. E.; Congreve, M. S.; Kay, C.; McKeown, S. C.; Murray, P. J.; Scicinski, J. J.; Watson, S. P. *Angew. Chem., Int. Ed.* **2000**, *39*, 3293–3296.
6. Scicinski, J. J.; Congreve, M. S.; Jamieson, C.; Ley, S. V.; Newman, E. S.; Vinader, V. M.; Carr, A. E. *J. Comb. Chem.* **2001**, *3*, 387–396.

7. Carrasco, M. R.; Fitzgerald, M. C.; Oda, Y.; Kent, S. B. H. *Tetrahedron Lett.* **1997**, *38*, 6331–6334.
8. Murray, P. J.; Kay, C.; Scicinski, J. J.; McKeown, S. C.; Watson, S. P.; Carr, R. A. E. *Tetrahedron Lett.* **1999**, *40*, 5609–5612.
9. McKeown, S. C.; Watson, S. P.; Carr, R. A. E.; Marshall, P. *Tetrahedron Lett.* **1999**, *40*, 2407–2410.
10. Wang, S. S. *J. Am. Chem. Soc.* **1973**, *95*, 1328–1333.
11. Phoon, C. W.; Oliver, S. F.; Abell, C. *Tetrahedron Lett.* **1998**, *39*, 7959–7962.
12. Hanessian, S.; Xie, F. *Tetrahedron Lett.* **1998**, *39*, 733–736.
13. Richter, L. S.; Gadek, T. R. *Tetrahedron Lett.* **1994**, *35*, 4705–4706.
14. Wang, Y.; Wilson, S. R. *Tetrahedron Lett.* **1997**, *38*, 4021–4024.
15. Boojamra, C. G.; Burow, K. M.; Ellman, J. A. *J. Org. Chem.* **1995**, *60*, 5742–5743.
16. Fivush, A. M.; Willson, T. M. *Tetrahedron Lett.* **1997**, *38*, 7151–7154.
17. Jensen, K. J.; Alsina, J.; Songster, M. F.; Vagner, J.; Albericio, F.; Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 5441–5452.
18. Sarantakis, D.; Bicksler, J. J. *Tetrahedron Lett.* **1997**, *38*, 7325–7328.
19. Swayze, E. E. *Tetrahedron Lett.* **1997**, *38*, 8465–8468.
20. Forns, P.; Sevilla, S.; Erra, M.; Ortega, A.; Fernandez, J. C.; de la Figuera, N.; Fernandez-Forner, D.; Albericio, F. *Tetrahedron Lett.* **2003**, *44*, 6907–6910.
21. Ankala, S. V.; Fenteany, G. *Tetrahedron Lett.* **2002**, *43*, 4729–4732.
22. Mitsunobu, O.; Yamada, M.; Mukayima, T. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 935–939.
23. When a sample of the polymer-supported linker **3** was treated with 10% TFA in DCM, LC/MS data indicated the presence of an ion 279 Da, corresponding to a chroman derivative **6**, rather than expected product **5**. Thus, the TBS mono-protected diol **5** was independently prepared from propane diol and TBS protected 4-hydroxybenzyl alcohol. Treatment of the alcohol **5** with 10% TFA in DCM led to the formation of the product, indistinguishable from the compound obtained by cleavage from the resin **3**. Experimental details will be published in a full report.



5



6

.
24. Ryoo, S. J.; Kim, J.; Kim, J. S.; Lee, Y. S. *J. Comb. Chem.* **2002**, *4*, 187–190.
25. Floyd, C. D.; Lewis, C. N.; Patel, S. R.; Whittaker, M. *Tetrahedron Lett.* **1996**, *37*, 8045–8048.
26. Gordeev, M. F.; Hui, H. C.; Gordon, E. M.; Patel, D. V. *Tetrahedron Lett.* **1997**, *38*, 1729–1732.
27. Flörsheimer, P.; Riniker, B. Solid-Phase Synthesis of Peptides with the Highly Acid-Sensitive HMPB Linker. In *Pept. 1990, Proc. Eur. Pept. Symp., 21st*; Giralt, E.; Andreu, D., Eds.; ESCOM: Leiden, 1991; pp 131–133.
28. Albericio, F.; Kneibcordonier, N.; Biancalana, S.; Gera, L.; Masada, R. I.; Hudson, D.; Barany, G. *J. Org. Chem.* **1990**, *55*, 3730–3743.