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A new bio-compatible pH cleavable linker for solid-phase synthesis of a squalamine analogue

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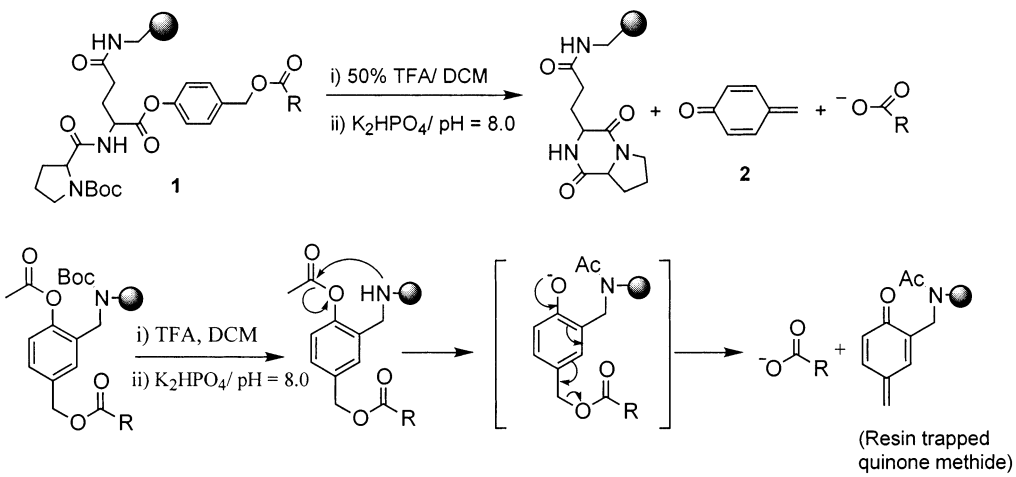
Abstract—Linkers that can be cleaved directly within the biological assay offer some advantages over traditional linkers in the range of direct screening applications that the associated libraries can be utilised for. The 1,6-elimination process is an efficient method of cleaving compounds from substituted 4-hydroxymethyl phenols, although giving rise to quinone methide by-products. Here, we report on a linker that uses an in-built amine 'activator' to cleave a phenoxy ester and hence to activate the linker to 1,6-elimination. An analogue of the antibacterial agent squalamine was synthesised and released using this linker strategy. © 2001 Elsevier Science Ltd. All rights reserved.

Linkers play a dominating role in solid-phase organic synthesis, determining not only the method of compound cleavage and attachment, but also placing restraints on the necessary nature of library synthesis.[1] Huge numbers of linkers are now known,[2] falling predominantly into electrophilically cleaved systems such as the Wang, Rink and trityl linkers,[3] and nucleophilically cleaved linkers such as the Kenner, Marshall and ester based linkers,[4] but also covering more exotic linkers such as light and thermally based cleavage processes.[5]

One linker type that has been used in the area of SPOS is the so-called 'safety catch linker'. These linkers require some form of preactivation prior to compound cleavage, often requiring two orthogonal steps to necessitate cleavage. Some of these linkers potentially allow compound cleavage to take place under mild and biocompatible conditions, either within assay wells or within agarose gels for zone diffusion-based screens. Such linkers have been described for example, by Frank[4] as well as by ourselves,[6] but problems with these linkers have limited their application. Here, we



Scheme 1. 1,6-Elimination process of pH cleavable linkers.

Keywords: solid-phase synthesis; linker; squalamine.

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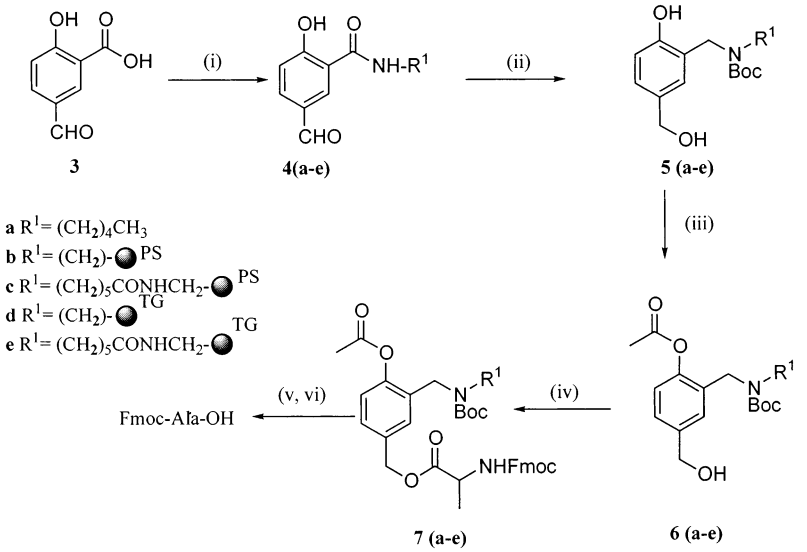
describe a new pH cleavable linker system, based on the 1,6-elimination process of our previously described linker **1**, but without liberation of the reactive quinone methide by-product **2** (Scheme 1).

The new linker model was initially prepared in solution (Scheme 2), followed by solid-phase attachment. The synthesis started with 5-formylsalicylic acid **3**, which was coupled to pentylamine to give **4a** (88%). This was then reduced with borane using the procedure described by Hall et al.[8] The amine was then protected without isolation with Boc_2O to give **5a**, followed by selective acetylation[9] of the phenol using the reagent 1-acetyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridine to give **6** (90%). Esterification of the hydroxymethyl entity with Fmoc-Ala-OH using DIC/DMAP gave **7a**[7] (86%). This was then used as a solution model for subsequent resin based chemistries.

The kinetics of cleavage of this linker in solution were monitored by RP-HPLC. The Boc protecting group was first removed by treating with a 50% solution of TFA/DCM followed by removal of volatiles and then treatment with K_2HPO_4 buffer (50 mM, pH 8.0). Samples were removed and quenched with 0.1% TFA before injection and quantification using internal standards. The production of Fmoc-Ala-OH and disappearance of starting material are shown in Fig. 1.

The half-life of cleavage was 12 minutes and Fmoc-Ala-OH was recovered in 96% yield. The linker was then synthesised directly on the solid-phase using both polystyrene and TentaGel resins. The resin was coupled directly with the building block **3** under standard reaction conditions to give **4** (**b–e**) (Scheme 2). The solution chemistry was repeated on the solid-phase to afford products **7** (**b–e**).

The kinetics of the release of Fmoc-Ala-OH from compound **7b** was then carried out in the same manner as described above. As expected, product release was slower than that obtained in solution and a half-life of 40 minutes was observed (Fig. 1). The kinetics of Fmoc-Ala-OH release from compound **7c** was identical



Scheme 2. Synthesis/cleavage of pH cleavable linker **7** (i) R^1NH_2, DIC, HOBt, DCM; (ii) a. BH_3·THF, 65°C; b. Boc_2O, DCM; (iii) 1 M NaOH (aq.), 1-acetyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridine, THF, rt; (iv) Fmoc-Ala-OH, DIC, DMAP, DCM; (v) 50% TFA/DCM, rt, 1 h; (vi) K_2HPO_4 buffer (50 mM), pH 8.0.

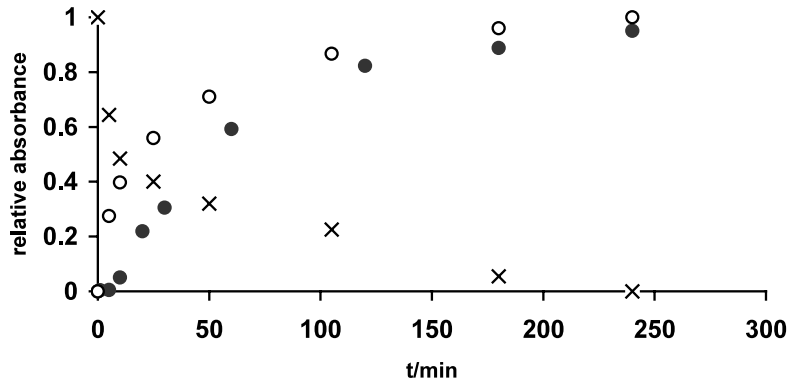
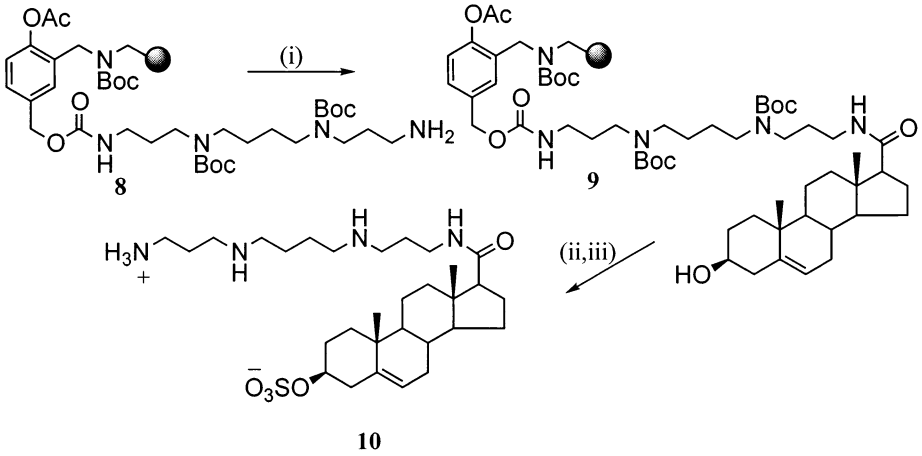


Figure 1. Kinetics of compound release from **7a** and **7b**: (x) disappearance of **7a**, (○) production of Fmoc-Ala-OH from **7a**, (●) production of Fmoc-Ala-OH from **7b**.



Scheme 3. *Reagents and conditions*: (i) 3β-acetoxybisnor-5-chlolenic acid/DIC/HOBt; (ii) Py·SO_3, $CHCl_3$; (iii) 50% TFA in DCM 1 h then phosphate buffer (pH 8.0) 24 h.

to that of **7b**. Hence the small hydrophobic spacer attached to the resin had no effect on the kinetics of cleavage. Other compounds released from the linker immobilised on PS included Fmoc-Phe-Ala-OH, Fmoc-Gly-Phe-Ala-OH and 4-hydroxy-7-trifluoromethyl-3-quinoline carboxylic acid. In all cases a quantitative recovery of the compounds released was achieved.

The linkers attached to TentaGel (compounds **7d** and **7e**) were found to cleave substantially upon treatment with acid to remove the amino protecting group. Use of various percentages of TFA (5–50%) and reaction times (1–30 min) to remove the Boc group still resulted in substantial cleavage of Fmoc-Ala-OH. An analogous structure to that of **7d** was synthesised, but using Bpoc instead of Boc as the amino protecting group. Here, after deprotection with acetic acid, most of the Fmoc-Ala-OH was released.

The intermediate **6c** was used in the synthesis of the antibacterial squalamine **10** (Scheme 3). Briefly, linker **6c** was transformed into an active carbonate using *p*-nitrophenyl chloroformate. The linker was then functionalised using diBoc-protected spermine to give compound **8**. The spermine template was coupled to 3β-acetoxybisnor-5-cholenic acid and compound **9** was converted to the sulfate derivative using sulfur trioxide in pyridine.[10] This compound was cleaved from the resin by activating with 50% TFA/DCM followed by treatment with pH 8.0 phosphate buffer (60% yield).

In summary, the synthesis of a new safety catch linker was achieved on the solid-phase. It was used for the first solid-phase synthesis of an analogue of the shark-derived antibacterial agent squalamine, and these linkers are suitable for cleaving directly within a biological assay. The utilisation of this linker for releasing transfection and antibacterial agents is under further investigation.

Acknowledgements

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7. Selected data for **7a**: δ_H (300 MHz, $CDCl_3$) 0.9 (3H, t, *J* 7, CH_3), 1.14–1.36 [6H, m, -$(CH_2)_3CH_3$], 1.36–1.6 (14H, m, Boc, -NCH_2 $(CH_2)_3CH_3$, and Ala-CH_3), 4.2 (1H, t, *J*

7, FmocCH), 4.3–4.5 [5H, m, $FmocCH_2$, $ArCH_2$-N, and AlaCH(α)], 5.17 (2H, Abq, *J* 7, $ArCH_2$), 5.45 (1H, d, *J* 7, NH), 7.06 (1H, d, *J* 8, Ar), 7.21–7.28 (2H, m, Ar), 7.32 (2H, t, *J* 7, Fmoc), 7.41 (2H, t, *J* 7, Fmoc), 7.65 (2H, d, *J* 7, Fmoc), 7.78 (2H, d, *J* 7, Fmoc); δ_C (75 MHz, $CDCl_3$) 14.41 (CH_3), 19.11 (CH_3-Ala), 21.30 (CH_3, Ac), 22.77 (CH_2CH_3), 27.98 ($CH_2CH_2CH_3$), 28.83 (Me_3), 29.38 [$CH_2(CH_2)_2CH_3$], 46.58 [N-$CH_2(CH_2)_3CH_3$], 47.58 (Fmoc-CH), 50.10 [Ala-CH(α)], 66.99 (Fmoc-CH_2, $ArCH_2$-N), 67.46 ($ArCH_2$), 80.10 (CMe_3), 120.39, 123.27, 125.51, 127.48, 128.12, 128.66, 130.05 (ArCH), 131.18, 133.63, 141.72, 144.20, 144.34, (ArC), 156.01 (urethanes), 169.56, 173.20 (esters); TLC R_f 0.32 (50:50 EtOAc:hexane); ν_{max} (CH_2Cl_2)/cm^{-1} 3541, 2963, 2952, 2923, 1760, 1726, 1691; ES-MS (+ve): *m*/*z* 676.7 $(M+NH_4)^+$, 681 $(M+Na)^+$, HRMS: expected for $C_{38}H_{46}N_2O_8$, 658.3332, found: 658.3358.

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