Molecular Diversity, **5:** 25–34, 2000.
KLUWER/ESCOM

A safety-catch linker for amine release under biologically compatible conditions

David Orain & Mark Bradley*
Department of Chemistry, University of Southampton, Southampton SO17 1BJ, U.K.
(To whom correspondence should be addressed)*

Received 15 May 2000; Accepted 18 September 2000

Key words: amines, diketopiperazine, solid phase organic synthesis (SPOS)

Summary

A 'biocompatible' safety-catch linker based on an internal diketopiperazine release has been developed to allow the release of amine based compounds. The synthesis of the linker and its pre-loading with a representative set of amines is described. After immobilization onto an amino Tentagel resin, the linker was activated and the amines were released under biological conditions at pH 8.

Introduction

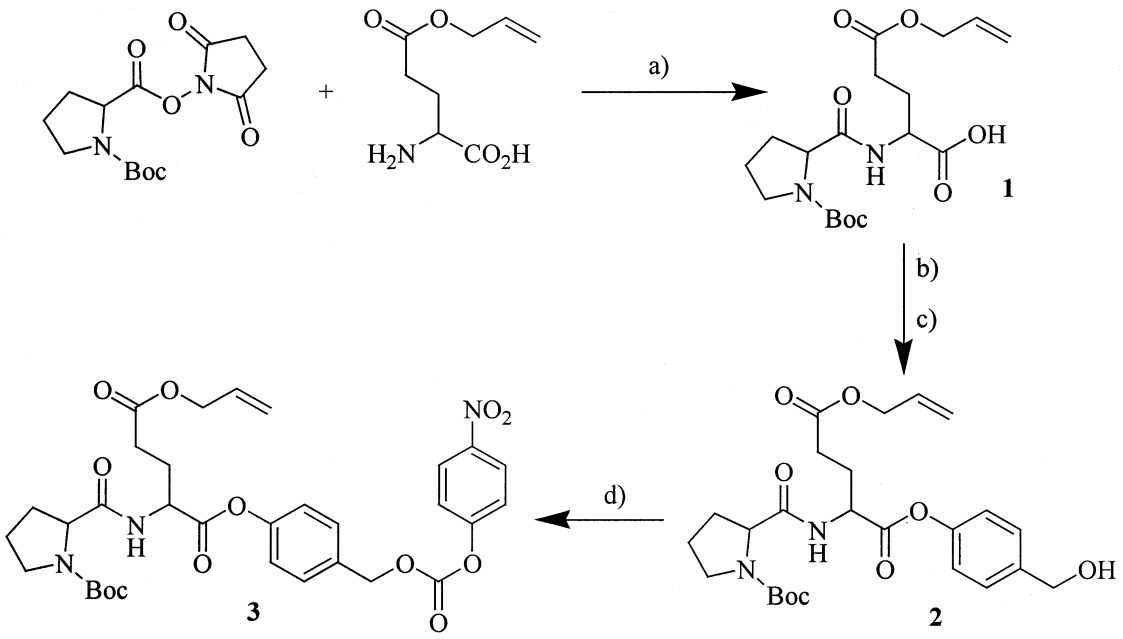
Over the last decade, solid phase synthesis and combinatorial chemistry have seen a dramatic evolution taking place [1]. Two strategies are now routinely applied in the production of thousands of compounds: parallel synthesis or split and mix synthesis. However, despite being a very efficient method, the split/mix strategy is used little, although potentially hundreds of thousands or millions of compounds can be rapidly and inexpensively prepared [2]. The rationale for this is threefold: firstly there are only tiny amounts of compound found on single beads, usually in the order of a few hundred picomoles; secondly the need to identify the compound on the bead and thirdly the lack of methods to enable the direct cleavage of compound from beads into assay wells under biologically compatible conditions (although a large range of linkers [3] have been reported, few of them could be described as 'biocompatible' [4] and in most cases compounds with acid functionalities are released).

As part of an ongoing program in this area, we have generated very high-loading beads [5] which solve the first part of this problem. Here, we report with the full synthetic details the synthesis of a linker that allows amine and amidine cleavage directly into the assay well ready for biological screening. This follows from a previous communication [6] that described a similar linker that was used to cleave carboxylic acids. Linker cleavage is driven by intra-molecular diketopiperazine formation [7] and the linker was prepared in solution and different amine scaffolds were pre-loaded on the linker before its attachment onto the resin.

Materials and methods

General

Solvents and reagents were obtained from commercial suppliers and used without further purification. Solid phase synthesis was performed on Tentagel-S-NH_2 from Rapp Polymer (0.29 mmol/g, 130 μm beads). All solid phase reactions were performed in glass peptide vessels or polypropylene syringes. Agitation of the reaction mixtures was achieved using a shaker or a rotative wheel. NMR spectra were recorded on a Bruker 400 MHz spectrometer with a deuterated solvent as the internal lock. ^{1}H NMR data are reported as: chemical shift (ppm), multiplicity, number of hydrogens, coupling constant. Analytical HPLC chromatograms were obtained on a Hewlett Packard HP-1100 system equipped with a Phenomenex Prodigy C_{18} reverse phase column (3.0 mm $\times$ 150 mm). Solvents used were: solvent A: 0.1% TFA in H_2O, solvent B: 0.042% TFA in CH_3CN, gradient 0% B to 100% B over 20 min. The column effluent was monitored us-



Scheme 1. Synthesis of the core part of the linker. Conditions: a) THF/H_2O (1/1), Et_3N 1.2 equiv, RT, 48 h (50–65%); b) 4-HO-C_6H_4-CHO, DCC, DMAP, DCM, 18 h, RT, (70–80%); c) $NaBH_4$, Green bromocresol, 20% HCl/EtOH (60–70%); d) 4-$NO_2C_6H_4$-OCOCl, Py, DCM, 15 min, RT (85–90%).

ing a variable wavelength UV detector at 220, 236 or 254 nm. Mass spectra were obtained on a VG Platform single quadrupole mass spectrometer under the electrospray positive ionization mode.

Synthesis

Synthesis of 2-[(1-tert-butoxycarbonyl-pyrrolidine-2-carbonyl)-amino]-pentanedioic acid 5-allyl ester **1**

Boc-Pro-OSuc (13.5 g, 43 mmol) and H-Glu(OAll)-OH (10.0 g, 53 mmol) were dissolved in water/THF (1/1, 150 mL). Triethylamine (8 mL, 57 mmol) was added dropwise and the mixture was stirred for 48 h at room temperature. THF was removed in vacuo and then the pH was adjusted to 2–3 with an aqueous solution of citric acid. The aqueous layer was extracted with AcOEt (3 × 150 mL). The organic layers were combined, washed with H_2O, dried over $MgSO_4$, filtered and then concentrated in vacuo. The resulting oil was purified by flash chromatography on silica gel to afford 8.2 g (50% yield) of **1** as a yellow oil. R_f: 0.18 (Hex/AcOEt/AcOH: 3.5/6/0.5). NMR ^1H ($CDCl_3$, 400 MHz): 9.22 (bs, 1H); 5.88 (ddt, 1H, J = 6, 10, 17 Hz); 5.31 (d, 1H, J = 16 Hz); 5.23 (d, 1H, J = 10 Hz); 4.58 (d, 2H, J = 6 Hz); 4.28 (bs, 1H); 3.44 (bm, 2H); 2.45 (m, 2H); 2.33–1.80 (m, 6H); 1.42 (s, 9H). NMR ^{13}C ($CDCl_3$, 100 MHz): 173.9; 172.3; 169.7; 155.0; 131.8; 118.5; 81.4; 65.5; 60.5; 59.6; 51.5; 47.0; 31.1; 30.0; 28.2; 26.9; 24.5; 23.6. M/z (ES+ve): 407.4 (20%) $[M+Na]^+$, 791.9 (100%) $[2M+Na]^+$.

Synthesis of 2-[(1-tert-butoxycarbonyl-pyrrolidine-2-carbonyl)-amino]-pentanedioic acid 5-allyl ester 1-(4-hydroxymethyl-phenyl) ester **2**

Compound **1** (10 g, 26 mmol) and 4-hydroxybenzaldehyde (3.5 g, 28.6 mmol) were dissolved in DCM (150 mL). DCC (5.9 g, 28.6 mmol) and DMAP (370 mg, 3 mmol) were added at 0 °C. The mixture was then stirred for 18 h at room temperature. The DCU was removed by filtration and the residue was concentrated in vacuo. The resulting oil was purified by flash chromatography on silica gel to afford the aldehyde (10.75 g, 85% yield) as a clear yellow oil.

The aldehyde (5.5 g, 11.3 mmol) was dissolved in MeOH (75 mL). A trace of bromocresol green was added to give a yellow colored solution. $NaBH_4$ (150 mg, 3.7 mmol) was added in small amounts. When $NaBH_4$ was added, the solution showed a blue color so a few drops of 20% HCl in EtOH were added to restore the yellow color. When the addition was over, the mixture was stirred for 30 min. The MeOH was removed in vacuo and the residue was taken up in water (150 mL). The aqueous layer was extracted with EtOAc (3 × 75 mL). The organic layers were com-

Table 1. Synthesis yields of compounds **7** and **8**

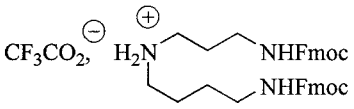
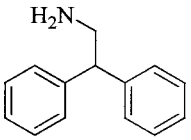
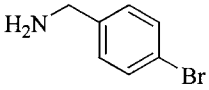
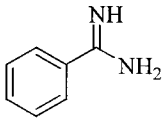
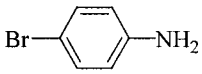
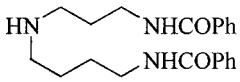
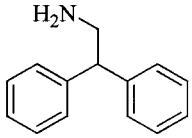
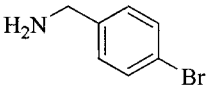
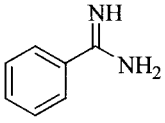
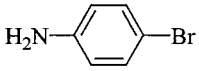
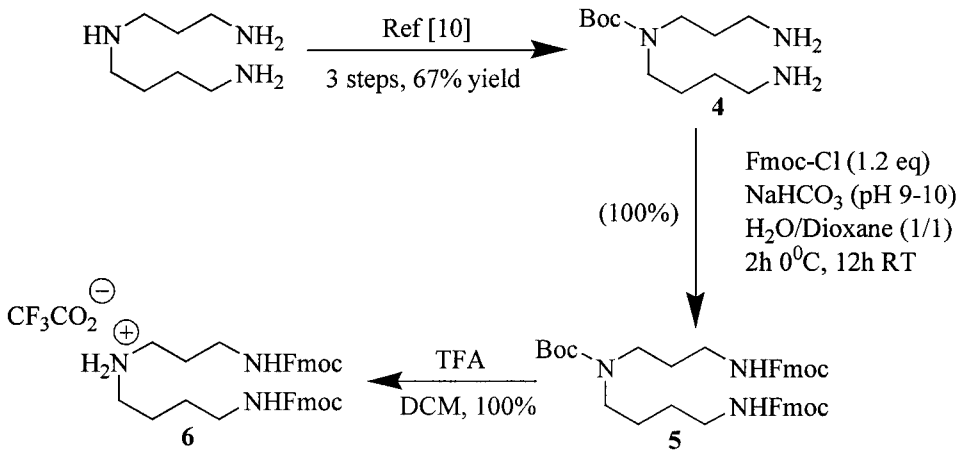
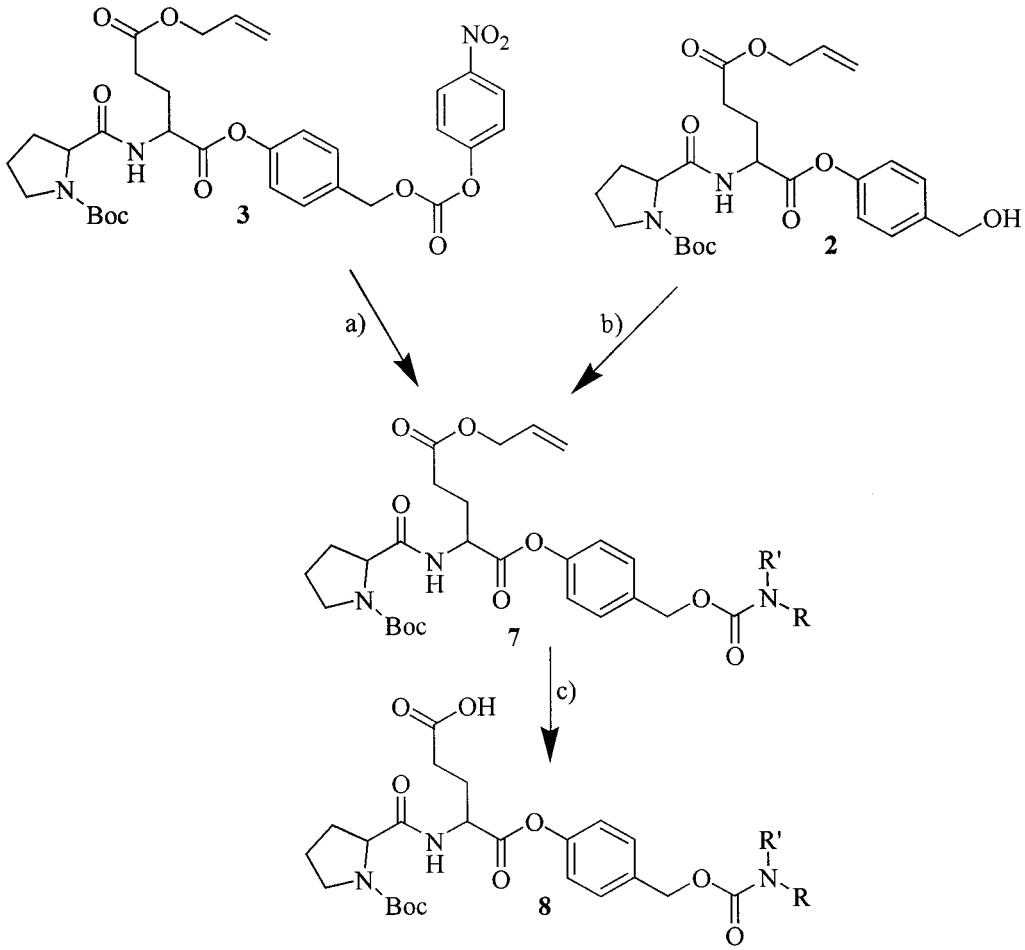
	R'RNH	**7** Yield	**8** Yield
a		50-55%	85%
b		80%	91%
c		69%	80%
d		73%	40%
e		77%	87%

Table 2. HPLC retention time of the released amines with their HPLC purities after first and second release

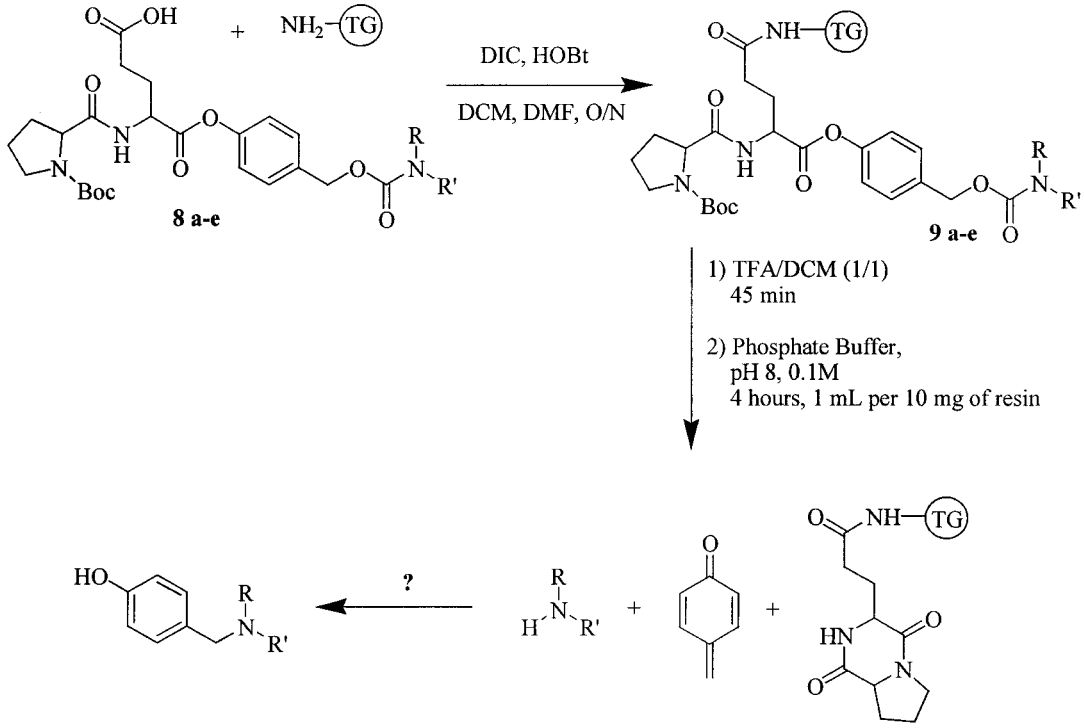
	R'RNH	**Retention Time (min)**	**Purity of 1st Release**	**Purity of 2nd Release**
a		7.4	95%	72%
b		7.5	70%	72%
c		6.6	45%	30%
d		5.4	81%	50%
e		6.6	71%	63%



Scheme 2. Formation N^1,N^8-Fmoc-spermidine salt **6**.



Scheme 3. Formation of the un-loaded linker **8e**. a) R′RNH (1.1 equiv), DIPEA (1 or 2 equiv), DMF, 2–4 h, RT, b) ArNCO (1.1 equiv), DIPEA (1.1 equiv), DMF, 1 h, RT, c) $Pd(PPh_3)_4$ (5% mol), pyrrolidine (1.5 equiv), DCM, 10 min, RT.



Scheme 4. The cleavage step.

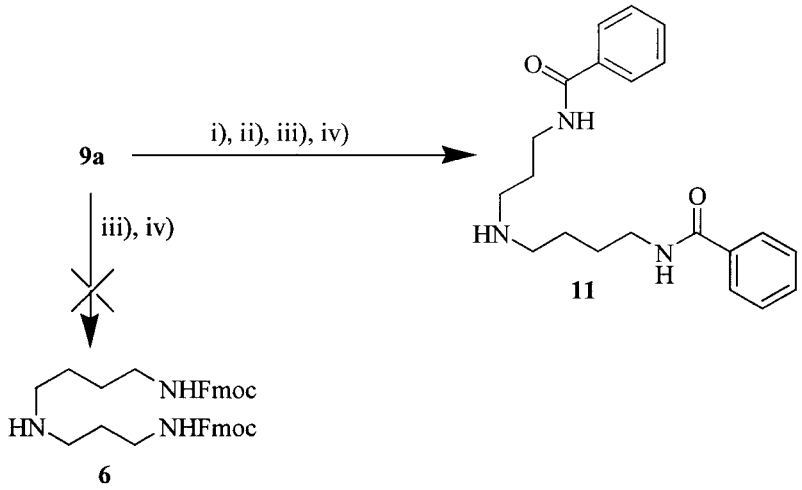
bined, washed with water, dried over $MgSO_4$, filtered, and then concentrated in vacuo. The resulting oil was purified by flash chromatography on silica gel to afford **2** (3.8 g, 63% yield) as a white–yellow sticky oil. R_f: 0.32 (AcOEt/PE: 85/15). NMR ^1H ($CDCl_3$, 400 MHz): 7.35 (d, 2H, J = 8.5 Hz); 7.16 (dd, 2H, J = 8.5, 2.0 Hz); 5.88 (ddt, 1H, J = 6, 10, 17 Hz); 5.30 (d, 1H, J = 17 Hz); 5.23 (d, 1H, J = 10 Hz); 4.75 (m, 1H); 4.66 (s, 2H); 4.58 (bd, 2H); 4.28 (bs, 1H); 3.40 (bm, 2H); 2.50 (m, 2H); 2.45–1.75 (m, 6H); 1.44 (s, 9H). NMR ^{13}C ($CDCl_3$, 100 MHz): 173.2; 172.3; 170.0; 155.7; 149.6; 139.0; 131.8; 128.0; 121.2; 118.4; 80.6; 65.5; 64.3; 60.9; 59.7; 51.7; 47.0; 30.9; 30.0; 28.3; 26.9; 24.6; 23.6. M/z (ES+ve): 491.4 (50%) $[M+H]^+$, 998.7 (100%) $[2M+NH_4]^+$.

Synthesis of 2-[(1-tert-butoxycarbonyl-pyrrolidine-2-carbonyl)-amino]-pentanedioic acid 5-allyl ester 1-[4-(4-nitro-phenoxycarbonyloxymethyl)-phenyl] ester **3**

Compound **2** (3.6 g, 7.3 mmol) was dissolved in DCM (50 mL). Pyridine (650 μL, 8 mmol) and 4-nitrophenylchloroformate (1.6 g, 8 mmol) were added. The mixture was stirred for 15 min at room temperature. The solvent was removed in vacuo and the resulting crude material was purified by flash chromatography on silica gel to afford **3** (4.65 g, 95% yield) as a yellow paste. R_f: 0.42 (AcOEt/PE: 1/1). NMR ^1H ($CDCl_3$, 400 MHz): 8.22 (d, 2H, J = 9 Hz); 7.44 (d, 2H, J = 8.5 Hz); 7.35 (d, 2H, J = 9 Hz); 7.12 (dd, 2H, J = 8.5, 3.0 Hz); 5.88 (ddt, 1H, J = 6, 10, 17 Hz); 5.29 (d, 1H, J = 17 Hz); 5.25 (s, 2H); 5.21 (d, 1H, J = 10 Hz); 4.75 (m, 1H); 4.57 (bd, 2H); 4.30 (bs, 1H); 3.40 (bm, 2H); 2.51 (m, 2H); 2.45–1.75 (m, 6H); 1.44 (s, 9H). NMR ^{13}C ($CDCl_3$, 100 MHz): 173.1; 172.2; 170.0; 155.7; 155.3; 152.2; 150.7; 145.2; 132.0; 131.8; 129.9; 125.1; 121.7; 118.3; 80.4; 70.0; 65.4; 60.8; 59.6; 51.6; 47.0; 30.9; 29.9; 28.2; 26.7; 24.5; 23.6. M/z (ES+ve): 656.4 (100%) $[M+H]^+$, 673.4 (50%) $[M+Na]^+$.

N^1,N^8-(fluorenylmethoxycarbonyl)-spermidine trifluoroacetate salt **6**

N^3-(tert-butyloxycarbonyl)-spermidine **4** (1.26 g, 5.15 mmol) was dissolved in H_2O/dioxane (1/1, 100 mL). The pH was adjusted to 9–10 by addition of $NaHCO_3$. At 0 °C, Fmoc-Cl (3.4 g, 13 mmol) was added in small fractions. The mixture was then stirred for 2 h at 0°C and 12 h at room temperature. Dioxane was removed in vacuo and the aqueous layer was



Scheme 5. i) 20% piperidine/DMF, ii) PhCOCl, pyridine, DCM, iii) TFA/DCM (1/1) 45 min, iv) phosphate buffer, pH 8, 0.1 M, 4 h.

extracted with AcOEt (3 × 50 mL). The organic layers were combined, washed with H_2O, dried over $MgSO_4$, filtered and then concentrated in vacuo. The resulting solid was purified by flash chromatography on silica gel to afford N^1,N^8-(fluorenylmethoxycarbonyl)-N^3-(tert-butyloxycarbonyl)-spermidine **5** (3.6 g, 100% yield) as a white solid. R_f: 0.50 (PE/AcOEt 1/1).

Compound **5** was dissolved in $CHCl_3$ (10 mL) and at 0 °C, TFA (7.7 mL, 20 equiv) was added dropwise. The mixture was stirred for 30 min at 0°C and then 2 h at room temperature The solvents were removed in vacuo and the resulting yellow paste was triturated with Et_2O to afford a white powder. This powder was washed with Et_2O and dried in vacuo. Compound **6** (3.6 g, 100%) was obtained as a white powder. NMR ^{1}H (DMSO-d_6, 400 MHz): 8.47 (bs, 2H); 7.87 (d, 4H, J = 7.5 Hz); 7.67 (d, 4H, J = 7.5 Hz); 7.41 (t, 4H, J = 7.5 Hz); 7.33 (t, 4H, J = 7.5 Hz); 4.40–4.20 (m, 6H); 3.05 (m, 4H); 2.87 (bs, 4H); 1.73 (m, 2H); 1.56 (m, 2H); 1.44 (m, 2H). NMR ^{13}C (DMSO-d_6, 100 MHz): 156.3; 156.1; 143.9; 140.8; 127.6; 127.0; 125.1; 120.1; 65.2; 46.8; 46.6; 44.6; 37.5; 26.4; 26.2; 22.9. M/z (ES+ve): 590.1 (100%) $[M+H]^+$.

General procedure for the synthesis of 2-[(1-tert-butoxycarbonyl-pyrrolidine-2-carbonyl)-amino]-pentanedioic acid 5-allyl ester 1-(4-R,R′-carbamoyloxymethyl-phenyl) ester **7a**–**d**

To a solution of **3** and the amine (1.1 equiv) in DMF was added DIPEA (2 or 3 equiv). The mixture was then stirred for 4 h. An equal volume of water was added and the aqeous layer was extracted with AcOEt. The organic layers were combined, washed with water, dried over $MgSO_4$, filtered, and then concentrated in vacuo. The resulting oil was purified by flash chromatography on silica gel to afford the urethanes **7a**–**d**.

Data for **7c**: NMR ^{1}H ($CDCl_3$, 400 MHz): 7.50–7.10 (m, 8H); 5.92 (ddt, 1H, J = 6, 10, 17 Hz); 5.33 (d, 1H, J = 17 Hz); 5.26 (d, 1H, J = 10 Hz); 5.12 (s, 2H); 4.80 (m, 1H); 4.61 (bd, 2H); 4.35 (m, 3H); 3.40 (bm, 2H); 2.54 (m, 2H); 2.45–1.80 (m, 6H); 1.48 (s, 9H). NMR ^{13}C ($CDCl_3$, 100 MHz): 172.2; 170.2; 170.0; 156.2; 150.1; 140.8; 134.3; 131.9; 130.4; 130.3; 130.2; 126.0; 122.6; 121.3; 118.4; 80.5; 66.1; 65.4; 60.9; 59.7; 53.3; 51.8; 51.6; 47.0; 44.4; 30.9; 30.0; 28.3; 26.9; 24.5; 23.6.

General procedure for the synthesis of 2-[(1-tert-butoxycarbonyl-pyrrolidine-2-carbonyl)-amino]-pentanedioic acid 1-(4-R′R′-carbamoyloxymethyl-phenyl) ester **8a**–**e**

To a degassed solution of **7a**–**e** in DCM, was added $Pd(PPh_3)_4$ (5% mol) under nitrogen. Pyrrolidine (1.5 equiv) was added dropwise and the mixture was stirred for 15 min at room temperature. The solvent was removed in vacuo and the resulting crude was purified by flash chromatography on silica gel to afford the corresponding acids **8a**–**e**.

Data for **8c**: NMR ^{1}H ($CDCl_3$, 400 MHz): 8.20 (bs, 2H); 7.50–6.90 (m, 8H); 5.11 (s, 2H); 4.70 (m, 1H); 4.34 (m, 3H); 3.50 (bm, 2H); 2.54 (m, 2H); 2.45–1.80 (m, 6H); 1.42 (s, 9H). NMR ^{13}C ($CDCl_3$, 100 MHz): 175.7; 170.2; 156.4; 156.0; 150.2; 140.6; 134.2; 130.6; 130.4; 130.2; 126.0; 122.7; 121.5; 82.5;

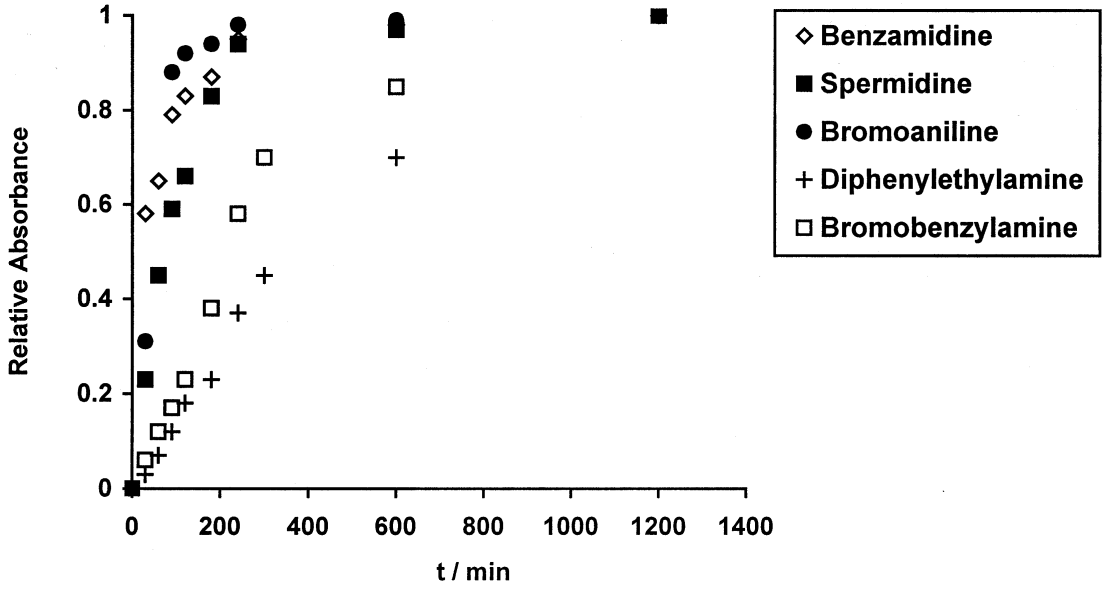


Figure 1. Kinetics of amine release from **9a**–**e**.

66.3; 60.9; 53.4; 52.7; 51.9; 47.0; 44.5; 31.2; 30.1; 28.3; 26.1; 25.7; 23.6.

General procedure for immobilisation of **8a**–**e** *on solid phase*

To a solution of acid (2 equiv) in DCM and a few drops of DMF (as necessary), HOBt (2 equiv) was added and the mixture was stirred for 10 min. DIC (2 equiv) was added and the solution was agitated for 10 min. The activated acid was then added to the resin preswollen in DCM and the mixture was shaken overnight. The resin was washed with DMF, DCM, MeOH and Et_2O and dried in vacuo. The completion of the coupling was verified by a qualitative ninhydrin test.

General procedure for the cleavage step

To the preswollen resin in DCM was added a solution of DCM/TFA (1/1) and the resin was shaken for 45 min. The resin was then washed with DCM, DMF, DCM, MeOH and water. A solution of sodium phosphate buffer (pH 8.00, 0.1 M) was added (1 mL per 10 mg of resin). The resin was shaken at room temperature for 4 h, filtered and the supernatant was analysed by HPLC.

Results and discussion

Scheme 1 shows the synthesis of the core part of the linker. The acid functionality of the side chain of glutamic acid, which will be the attachment point onto the resin, was protected with an allyl ester group. Reaction between the activated ester Boc-Pro-OSuc and the glutamic derivative H-Glu(OAll)-OH [8] in THF/water in the presence of Et_3N [9] provided **1** in an isolated yield of 50–65%. Esterification with 4-hydroxybenzaldehyde and subsequent reduction of the aldehyde with sodium borohydride under pH controlled conditions, led to the corresponding benzylic alcohol **2**. In order to form future urethane bonds, **2** was transformed into the activated carbonate **3** by reaction with 4-nitrophenylchloroformate in DCM in the presence of pyridine.

At this point, the core structure of the linker is intact and a range of amines or amidine can be introduced by displacement of the nitro phenol group. We focused on a representative set of amines: primary, secondary, benzylic, aromatic and benzamidine, and included the spermidine scaffold N^1,N^8-bis-Fmoc-spermidine. This was obtained in a very high yield by a five-step procedure starting from unprotected spermidine, via N^3-(tert-butoxycarbonyl)-spermidine **4** [10], with Fmoc introduction being achieved quantitatively with Fmoc-Cl. Treatment of **5** with TFA in DCM led to the formation of the N^1,N^8-Fmoc-spermidine salt **6** (Scheme 2). This compound was retained as its salt because the free amine obviously promoted Fmoc deprotection.

Reaction of **3** with the different amines in DMF in the presence of 2 or 3 equiv of DIPEA led to the form-

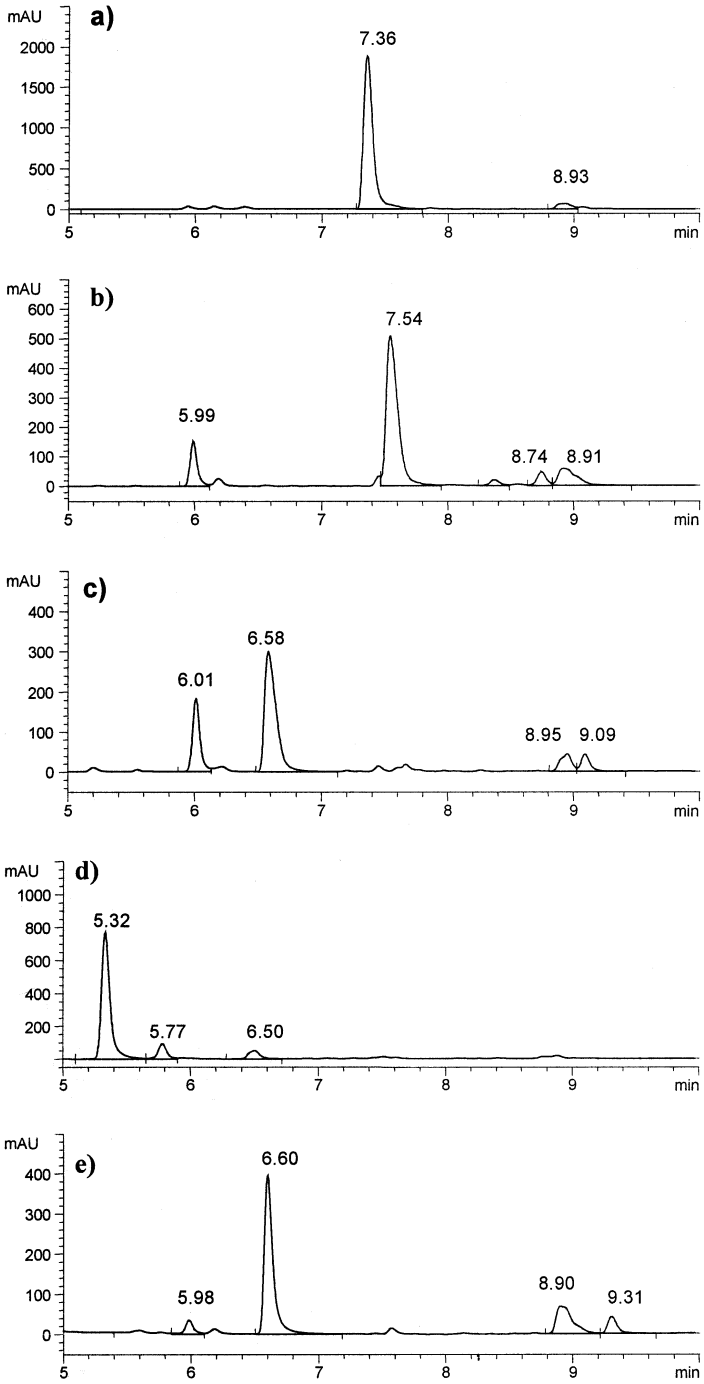


Figure 2. HPLC traces of the released amines (first release): (**a**) Bis-benzoyl-spermidine, (**b**) diphenylethylamine, (**c**) bromobenzylamine, (**d**) benzamidine, (**e**) bromoaniline.

ation of the corresponding urethanes **7a**–**d** in relatively good yields (Table 1). For the condensation of **6**, slow addition of DIPEA gave the best results. Subsequent deprotection of the allyl ester with palladium tetrakis triphenylphosphine in the presence of pyrrolidine as scavenger, afforded the corresponding acids **8a**–**d** (Table 2).

Aniline proved too un-reactive with **3** and thus a different strategy was used to create the urethane bond between the linker and aromatic amine. Thus, the aromatic isocyanate 4-bromophenylisocyanate was reacted with **2** in the presence of DIPEA in DMF to afford the corresponding aromatic urethane **7e** in 77% yield. Deprotection of the side chain was performed as usual to give the un-loaded linker **8e** containing the aniline (Scheme 3).

The acids **8a**–**e** were activated with HOBt and DIC and then immobilised onto aminomethyl Tentagel resin due to its compatibility with water, which will be the solvent of the cleavage step. Completion of the coupling reaction was followed by a ninhydrin test and 2 equiv of acid were used. In the case of immobilisation of the spermidine scaffold, an Fmoc quantitative test was carried out and gave the expected value of loading (0.23 mmol/g). The resulting resins were then submitted to the cleavage step. First, the safety catch-linker was activated by treatment with a solution of TFA in DCM (1/1) and then the resin was washed with DCM, DMF, DCM, MeOH and H_2O. The activated resins were shaken in a buffer solution (0.1 M of sodium phosphate buffer, pH 8.00) for 4 h [7] (Scheme 4).

Typically, after activation of the safety-catch linker and treatment with the buffer, the solution was recovered by filtration and analysed by HPLC and compared with authentic material.

For the release of diphenylethylamine, benzamidine and bromoaniline, good levels of purity of the recovered amines were achieved and no re-addition to the quinone methine was observed.

Only for the release of bromobenzylamine was the purity low (45% by HPLC). The major impurity showed a retention time of 6.00 min with nearly the same intensity of the amine. Unfortunately, we were not able to determine the structure of this secondary peak. Different buffers in a pH range of 6.50 to 8.50 were tried, but no significant change was observed in the ratio of two peaks. The peak at 6.00 min was also observed in the HPLC traces during other amine releases but in a very low concentration, suggesting it is some component of the linker.

In the case of the cleavage of **9a**, we never observed any bis-Fmoc-spermidine **6** by HPLC. This was probably due to the basicity of the system, which resulted in Fmoc degradation. To overcome this problem, the amino groups were capped with benzoyl chloride after Fmoc deprotection. Completion of the coupling was followed by a qualitative ninhydrin test. After activation with TFA, the resin was treated with buffer and the supernatant was analysed by HPLC and showed a single major peak (95%) corresponding to **11** (Scheme 5).

The cleavage rate was studied for the five released amines and followed by HPLC analysis. The complete release was achieved after 4 h for the benzamidine, bromoaniline and spermidine compounds. For the case of the diphenylethylamine and the bromobenzylamine, the release was much slower, probably due to low solubility of these compounds in water (Figure 1).

All the resins were filtered off and submitted to buffer for a second time (4 h). All the resins again released amine, showing the relatively slow cleavage of compound, although the relative absorbance was much reduced compared to the first release (between 10 and 40%) and generally the purity of the released amine during the second run was inferior.

The purity of the released amines is reported in Table 2 and Figure 2 shows the HPLC of the first five releases.

Conclusions

In summary, we have developed an efficient synthesis of a safety-catch linker for the release of amines under direct assay conditions. A large range of amines or isocyanates could be introduced onto this linker. We have shown that this linker allows the release of primary, secondary aliphatic amines, arylamines and benzamidine under neutral conditions. In addition, due to the kinetics of cleavage, this linker can be used to allow multiple release. We are currently investigating the synthesis of inhibitors of the trypanothione reductase [11] on this linker containing the spermidine scaffold and its application to direct screening methods.

Acknowledgements

We thank the BBSRC (51/BO9512) for funding.

References

1. a. Terret, N.K., Gardner, M., Gordon, D.W., Kobylecki, R.J. and Steele, J., *Combinatorial synthesis – The design of compound libraries and their application to drug discovery*, Tetrahedron, 51 (1995) 8135–8173.
   b. Gordon, E.M., Gallop, M.A. and Patel, D.V., *Strategy and tactics in combinatorial organic synthesis. Applications to drug discovery*, Acc. Chem. Res., 29 (1996) 144–154.
   c. Lam, K.S., Lebl, M. and Krchnák, V., *The 'one-bead-one-compound' combinatorial library method*, Chem. Rev., 97 (1997) 411–448.
2. a. Still, W.C., *Discovery of sequence-selective peptide binding by synthetic receptors using encoded combinatorial libraries*, Acc. Chem. Res., 29 (1996) 155–170.
   b. Lam, K.S., Salmon, S.E., Hersh, E.M., Hruby, V.J., Kazmierski, W.M. and Knapp, R.J., *A new type of synthetic peptide library for identifying ligand-binding activity*, Nature (London), 354 (1991) 82–84.
   c. Meldal, M., Svendsen, I., Breddam, K. and Auzanneau, F.-I., *Portion-mixing peptide libraries of quenched fluorogenic substrates for complete subsite mapping of endoprotease specificity*, Proc. Natl. Acad. Sci. USA, 91 (1994) 3314–3318.
   d. Youngquist, R.S., Fuentes, G.R., Lacey, M.P. and Keough, T., *Generation and screening of combinatorial peptide libraries designed for rapid sequencing by mass spectroscopy*, J. Am. Chem. Soc., 117 (1995) 3900–3906.
3. a. James, I.W., *Linkers for solid phase organic synthesis*, Tetrahedron, 55 (1999) 4855–4946.
   b. Guillier, F., Orain, D. and Bradley, M., *Linkers and cleavage strategies in solid-phase organic synthesis and combinatorial chemistry*, Chem. Rev., 100 (2000) 2091–2157.
4. a. Salmon, S.E., Lam, K.S., Lebl, M., Kandola, A., Khattri, P.S., Wade, S., Pátek, M., Kocis, P., Krchnák, V., Thorpe, D. and Felder, S., *Discovery of biologically active peptides in random libraries: solution-phase testing after staged orthogonal release from resin beads*, Proc. Natl. Acad. Sci. USA, 90 (1993) 11708–11712.
   b. Kočiš, P., Krchňák, V. and Lebl, M., *Symmetrical structure allowing the selective multiple release of a defined quantity of peptide from a single bead of polymeric support*, Tetrahedron Lett., 34 (1993) 7251–7252.
   c. Pátek, M. and Lebl, M., *Safety-catch and multiply cleavable linkers in solid phase synthesis*, Biopolymers, 47 (1998) 353–363.
5. a. Fromont, C. and Bradley, M., *High-loading resin beads for solid-phase synthesis using triple branching symmetrical dendrimers*, Chem. Commun., (2000) 283–284.
   b. Wells, N.J., Basso, A. and Bradley, M., *Solid-phase dendrimer synthesis*, Biopolymers, 47 (1998) 231–396.
   c. Swali, V., Wells, N.J., Langley, G.J. and Bradley, M., *Solid-phase dendrimer synthesis and generation of super-high-loading resin beads for combinatorial chemistry*, J. Org. Chem., 62 (1997) 4902–4903.
6. Bray, A.M., Maeji, N.J., Valerio, R.M, Campbell, R.A. and Geysen, H.M., *Direct cleavage of peptides from solid support into aqueous buffer. Application in simultaneous multiple peptide synthesis*, J. Org. Chem., 56 (1991) 6659–6666.
7. Atrash, B. and Bradley, M., *A pH cleavable linker for zone diffusion assays and single bead solution screens in combinatorial chemistry*, Chem. Commun., (1997) 1397–1398.
8. Belshaw, P.J., Mzengeza, S. and Lajoie, G.A., *Chlorotrimethylsilane mediated formation of ω-allylesters of aspartic and glutamic acids*, Synth. Commun., 20 (1990) 3157–3160.
9. Nakatami, M., Nakata, T., Kouge, K. and Okei, H., *Studies on bitter peptides from casein hydrolyzate XIV Bitter taste of synthetic analogs of octapeptides, Arg-Gly-Pro-Phe-Pro-Ile-Ile-Val, corresponding to the C-terminal portion of β-casein*, Bull. Soc. Chim. Jap., 67 (1994) 438–444.
10. An, H., Cummins, L.L., Griffey, R.H., Bharadwaj, R., Haly, B.D., Fraser, A.S., Wilson-Lingardo, L., Risen, L.M., Wyatt, J.R. and Cook, P.D., *Solution phase combinatorial synthesis. Discovery of novel polyazapyridinophanes with potent antibacterial activity by a solution phase simultaneous addition of functionalities approach*, J. Am. Chem. Soc., 119 (1997) 3696–3708.
11. a. Smith, H.K. and Bradley, M., *Comparison of resin and solution screening methodologies in combinatorial chemistry and the identification of a 100 nM inhibitor of trypanothione reductase*, J. Comb. Chem., 1 (1999) 326–332.
    b. Marsh, I.R. and Bradley, M., *Solid phase synthesis of polyamine conjugates for the study of trypanothione reductase*, Tetrahedron, 53 (1997) 17317–17334.