QSAR & Combinatorial Science

A Re-evaluation of the Use of Rink, BAL, and PAL Resins and Linkers

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Full Paper

The preparation of amine and guanidine derivatives of phenylalaninamide and tryptophanamide as well as benzylamines from BAL, Rink-MBHA, and Rink resins has been performed. Cleavage of the target compound gave significant amounts of byproducts compounds in which the linker moiety was attached to the target unit. This side reaction can be avoided when the solid supports are prepared by anchoring the corresponding linker to an aminomethyl resin.

1. Introduction

The solid-phase synthesis of both biomolecules and small molecules starts by anchoring the first building block (BB) to the solid support. This can be performed directly onto a *functionalized solid support* [e.g. on a chloromethyl polystyrene (Merrifield) resin] or, alternatively, through a *linker/handle* that is incorporated to a solid support [1, 2]. Most of the linkers are bifunctional spacers that incorporate on one end features of a selectively removable protecting group (b, Figure 1) and a second end that serves to achieve the required anchoring to the resin as a separate chemical step (a, Figure 1) [3].

The success of any solid-phase approach using linkers/handles is dependent on the *permanent* (a) anchoring being stable during all the synthetic processes including the final cleavage of the *temporary* (b) anchoring.

In 1984 one of us described PAL (**P**eptide **A**mide **L**inker) (**1**) [4], which was the first linker for the solid-phase synthesis of peptide amides by the Fmoc/*t*Bu strategy. A few years later, Rink described the Rink resin (**2**) for the preparation of the same kind of peptides [5, 6], which was followed by the AM or Rink linker (**3**) for the same purpose [7]. Furthermore, BAL (**B**ackbone **A**mide **L**inker) (**4**) [8], which is the aldehyde precursor of PAL, has been used for the preparation of hundreds of *C*-terminal modified peptides, heterocycles, and other small organic molecules –

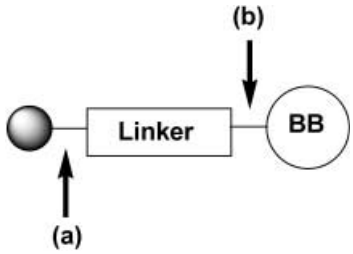

Figure 1. Solid-phase strategy when handles/linkers (*grafted linker* according to Bradley terminology [1b]) are used. (a): *permanent* anchoring; (b) *temporary* anchoring

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Key words: Combinatorial Chemistry, Handle, AM Linker, Side-reaction, Solid-phase

Abbreviations: Abbreviations used for amino acids and the designations of peptides follow the rules of the IUPAC-IUB Commission of Biochemical Nomenclature in *J. Biol. Chem*. **1982**, *247*, 977-983. The following additional abbreviations are used: BAL, backbone amide linker; *t*-Bu, *tert*-butyl; DCM, methylene chloride; DIEA, *N,N,*-diisopropylethylamine; DIPCDI, *N,N′*-Diisopropylcarbodiimide; DMF, *N,N*-dimethylformamide; Fmoc, 9-fluorenylmethyloxycarbonyl; HOBt, 1-hydroxybenzotriazole; MBHA, *p*-methylbenzhydrylamine resin; MeOH, methanol; PAL, 5-[4-(9-fluorenylmethyloxycarbonyl)aminomethyl-3,5-dimethoxyphenoxy]valeric acid; TFA, trifluoroacetic acid; TIS, triisopropylsilane; THF, tetrahydrofuran; TMOF, trimethyl orthoformate; XAL, xanthenylamide linker.

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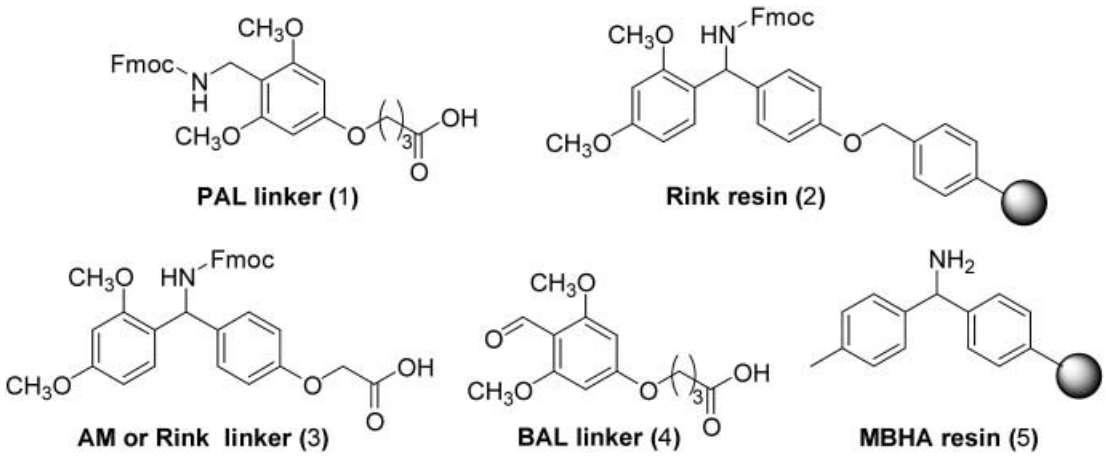


Figure 2. Linkers and resins used for the preparation of amides and amines (5 is an example of a *functionalized solid support* or *integral linker*)

always through amide/peptide bond anchoring [8, 9]. This approach involves the attachment of an amine nitrogen by reductive amination and further acylation. More recently, Rink [10] and BAL [11] based resins have been used for the preparation of benzyl and secondary amines, respectively. PAL, Rink, and BAL systems are usually incorporated onto MBHA resin (**5**) [12, 13] through an amide bond. The use of all these linkers and resins allows the preparation of amides and amines by treatment with acid solutions.

Results and Discussion

Several of our current programs in *Solid-Phase Combinatorial Chemistry* have been focused on the preparation of libraries using an aromatic amino acid, either Trp (40 compounds in total) or Phe (18 compounds in total), as scaffolds as shown in Figure 3.

Compounds **6** were prepared using either a BAL-MBHA resin (where R_1 is an alkyl or a substituted benzyl group) or a Rink-MBHA resin (where R_1 is H). Compounds **7**, on the other hand, were prepared using a Rink resin. All compounds bearing acyl, sulfonyl and Fmoc-protected amines as the X/Y substituents gave good yields and purities regardless of the structure and the solid support used.

However, when compounds bearing amines, ammonium salts or guanidines were released from the resins with TFA/TIS/H_2O (90:5:5) (BAL and Rink-MBHA resins) or TFA/DCM (25:75) (Rink resin) for 2 h at 25 °C, the target compound were accompanied by side-products with mo-

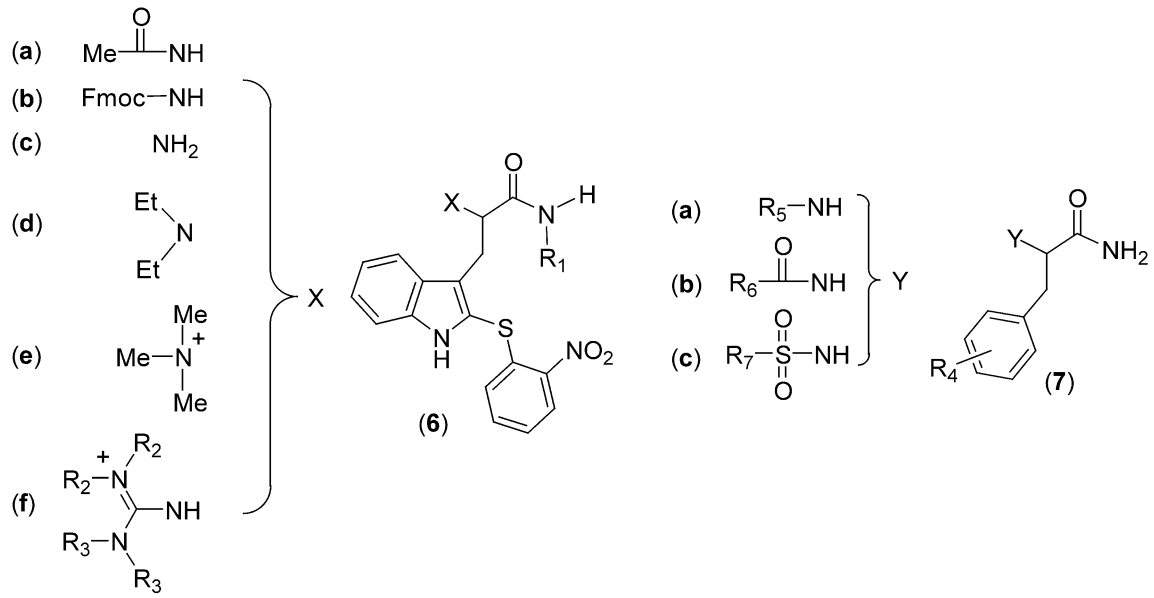


Figure 3. Amino amides considered in the present study.

lecular weights of +251 [4] (for BAL-MBHA resin synthesis), +299 (for Rink-MBHA resin synthesis) or +243 (for Rink resin synthesis). These correspond to the target compound attached to the BAL linker amide (**8**), to the Rink linker amide (**9**), or to the Rink moiety (**10**), respectively. The extent of byproducts formation depends on the nature of the pendant groups (X or Y, Figure 3) and the solid support [14].

For the series (**6**) with BAL-MBHA resin, side-products (**8c–f**) were obtained with different purities. The purities were as follows; 70–80% for the trimethylammonium synthesis (**6e**, 4 compounds corresponding to 4 different R_1); an equimolar proportion with respect to the target compound for the diethylamine derivatives (**6d**, 4 compounds); 10–15% for guanidine derivatives [**6f**, 16 compounds (4 guanidines $\times$ 4 R_1)] and primary amines (**6c**, 4 compounds) (Table 1).

For series **6**, prepared with Rink-MBHA resin, side-products (**9c–f**) were obtained as the major components (75–80%) in the diethylamine and triethylammonium derivatives (**6d, e**) and in an equimolar amount with respect to the target compound for the primary amines (**6c**) and one series of guanidine derivatives (**6f**). Finally, for three series of guanidine derivatives (total of 12 compounds) the target compound was the major product (Table 1).

The side-products **10a** were obtained in the range of 20–40% with respect to the desired products **7a** upon cleavage from the Rink resin.

A few precedents have been reported in the literature for the unexpected cleavage of linker from resins [4c, 15, 16]. In one of these cases [4c], Trp-containing peptides were prepared from PAL-MBHA resins and, in this case, the PAL linker was detached from the MBHA resin and alkylated the peptide through the Trp residue [17]. This side reaction was totally avoided when PAL was attached onto the N^{α}-amino group of an amino acid attached to the MBHA resin [18]. On the basis of this information, Rink and BAL resins were prepared by attachment the linkers onto an MBHA resin containing a residue of Ala and the occurrence of the side-reaction was studied by undertaking the preparation of some benzylamine derivatives as models.

When benzylamines were prepared from Rink-MBHA resin (no amino acid), the final TFA treatment gave in some cases the target compound (**12**) along with two byproducts (see Table 2, entries 1–7). One byproduct was the Rink handle amide (**13**) (m.w. 299) and the second one was found to have a m.w. of the desired amine +299, which corresponds to the Rink linker amide attached to the target compound (**14**). The side reaction is more important when stronger electron-donating groups are present on the aromatic ring of the benzylamine (see Table 2, entries 4–7 *vs* 1–3).

Although the presence of the amino acids (entries 8–13, Table 2) led to a slight decrease in the formation of byproducts (**15**, **16**), they are still present in the final crude material. Regardless of the presence of the amino acids, the extent of the side reaction is greater when a high concentration of TFA is used. When the concentration of TFA is around 15–30% the side-reaction either does not take place or takes place to only a small extent. However, in that case, the cleavage yield of the target compound from the resin is also very low and therefore such conditions are not suitable for a combinatorial chemistry program [19].

An attempt was made to identify the byproduct, compound **16b** (entry 13, Table 2), and two possible structures can be postulated: **16b1**, where the benzylamine is directly attached to Rink-Ala-NH_2 through the benzyl position of the linker, and **16b2**, where the linker has suffered back alkylation by the benzylamine through any position of the aromatic ring. This latter structure is similar to that found by one of us during the synthesis of Trp-containing peptides with a PAL resin [17].

The crude material, which was identified as a single peak by LC-MS with a *m/z* of 523, was characterized by ^{1}H-NMR, ^{13}C-NMR, HSQC, COSY and TOCSY (see additional information for spectra) and the results are consistent with structure **16b1.**

The results outlined in Table 2 were corroborated when Rink-Ala-MBHA resin was used. In this case, cleavage with

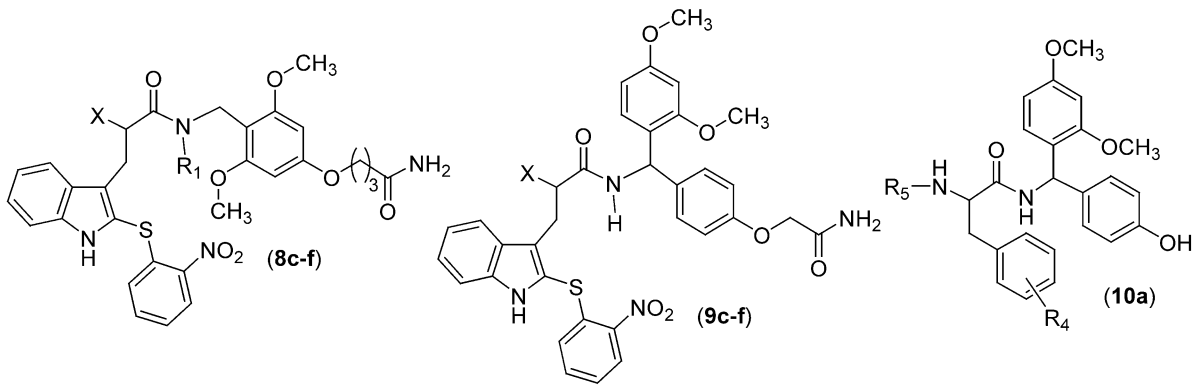
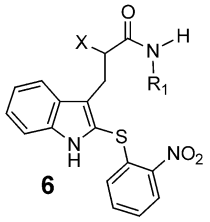
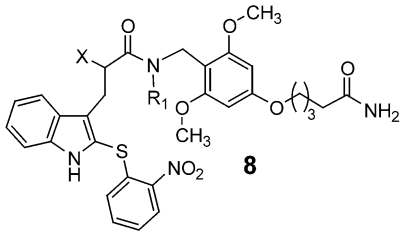
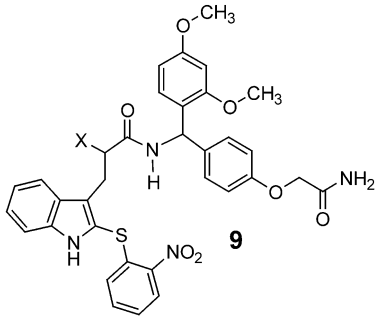
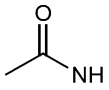
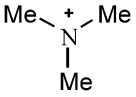
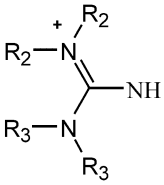


Figure 4. Side-products released from Trp analogs anchored to BAL- and Rink-MBHA resins and from Phe analogs anchored to Rink resin.

Table 1. HPLC analysis for TFA cleavage of Trp derivatives bound to BAL- and Rink-MBHA resins

X	Resin	 **6**	 **8**	 **9**
a 	BAL	80–85%	–	NA*
	Rink	87%	NA	–
b Fmoc—NH	BAL	85–90%	–	NA
	Rink	90%	NA	–
c NH_2	BAL	40–60%	10–15%	NA
	Rink	55%	NA	30%
d 	BAL	40–50%	30–40%	NA
	Rink	15%	NA	64%
e 	BAL	5–15%	70–80%	NA
	Rink	10%	NA	85%
f 	BAL	30–60%	10–15%	NA
	Rink	45–60%	NA	30–40%

* Not applicable

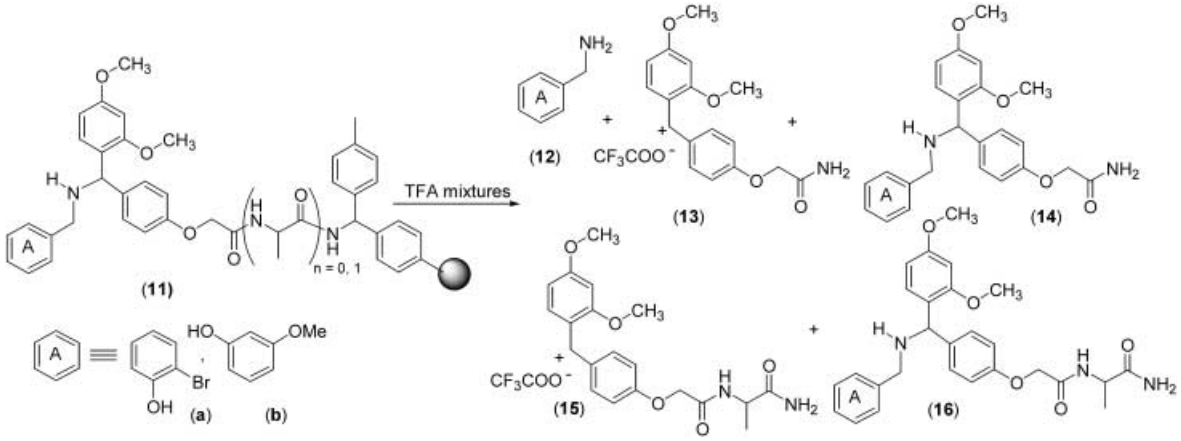
neat TFA gave Rink-Ala-NH_2 (**16**) as the major product. Similar results were obtained when 2-hydroxy-5-methoxybenzylamine was released from an XAL-MBHA resin [20], wherein the cleavage mixture contained **12b** (68%), XAL-NH_2 (similar to **13**, 12%) and **12b**-XAL-NH_2 (similar to **14**, 14%).

In the same way, the synthesis of Trp (**6**) and Phe (**7**) derivatives was carried out with Ala as a linker amino acid on an MBHA resin. Thus, when **6a–c** ($R_1 = H$) were released from Rink-Ala-MBHA resin, the acyl (**6a**) and Fmoc (**6b**) derivatives were obtained in good yields and purities – as in the synthesis carried out on Rink-MBHA resin (no Ala). However, **6c** was obtained together with **6c**-Rink-Ala-NH_2 (equimolar amounts) when the cleavage mixtures contained TFA percentages greater than 50%, while lower concentrations of TFA (15%) did not give any product [21].

Furthermore, the release of **7a** from Rink-Ala-MBHA resins with TFA/DCM (95:5) gave similar results to those described above. Indeed, the crude mixture contained **7a** (7%), **7a-**Rink-handle-Ala-NH_2 (56%) and Rink-handle-Ala-NH_2 (23%).

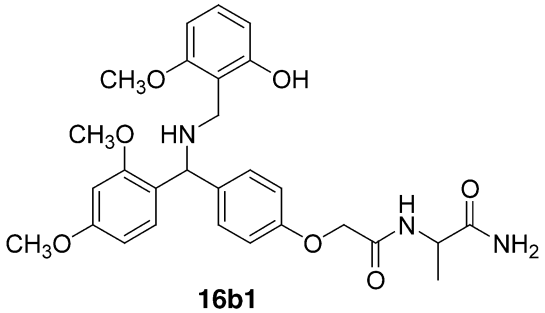
A common characteristic of all substrates that give rise to this side reaction is that they contain a basic center (amine or guanidine) close to the linker. The presence of this basic center is a prerequisite for the side reaction to occur, because when the free amine is acylated or sulfonated the reaction does not occur at all. In the cases of compounds **6**

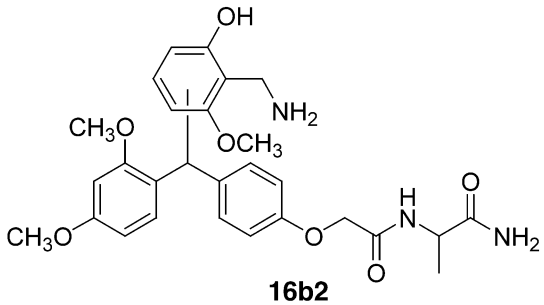
Table 2. HPLC analysis of the TFA cleavage of amine-bound resins (11)



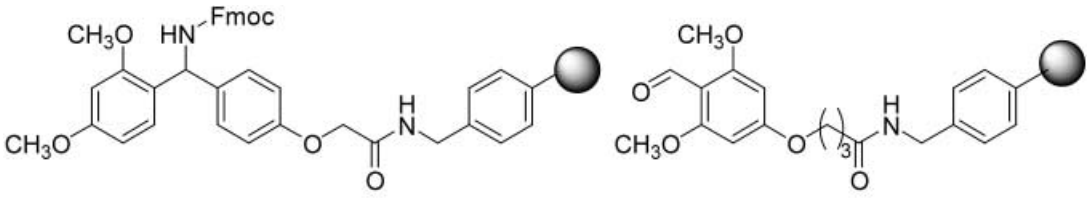
Entry #	n	Amine-Bound Resin	TFA cleavage mixture*	**12** (%)	**13** (%)	**14** (%)	**15** (%)	**16** (%)
1		**11a**	TFA/TIS/H_2O/DCM (15:5:5:75)	100	–	–		
2		**11a**	TFA/TIS/H_2O/DCM (30:5:5:60)	100	–	–		
3		**11a**	TFA/H_2O (95:5)	54	43	–		
4	0	**11b**	TFA/TIS/H_2O/DCM (5:5:5:85)	60	–	–		
5	No Ala	**11b**	TFA/TIS/H_2O/DCM (15:5:5:75)	54	36	–		
6		**11b**	TFA/TIS/H_2O/DCM (30:5:5:60)	45	34	21		
7		**11b**	TFA/H_2O (95:5)	–	50	50		
8		**11a**	TFA/TIS/H_2O/DCM (15:5:5:75)	93	–	–	4	–
9		**11a**	TFA/TIS/H_2O/DCM (30:5:5:65)	100	–	–	–	–
10	1 with Ala	**11a**	TFA/TIS/H_2O/DCM (50:5:5:40)	75	–	–	15	–
11		**11a**	TFA/H_2O (95:5)	59	–	–	10	–
12		**11a**	TFA (100)	53	–	–	28	–
13		**11b**	TFA (100)	–	–	–	–	93

* Yields were very low when low concentrations (5–30%) of TFA were used.





and **7**, the presence of the positive charge at the α-nitrogen decreases the basicity of the oxygen of the carboxyl group and, therefore, increases the stability of the amide bond that binds the compound to the resin. Similarly, amines are difficult to release from polyalkoxybenzyl-based resins [22]. In both cases, the amide bond between the linker or Ala and the MBHA resin [for BAL/Rink-(Ala)-MBHA resin] or the benzyl phenyl ether (for Rink resin) is perhaps of a similar acid lability to the bond between the compound and the resin. This situation implies that cleavage can occur at both sites. As the problem mainly concerns the lability of the permanent anchoring [(a) in Figure 1], an aminomethyl resin was used to anchor the handle to the solid support [23]. In this case, products derived from cleavage between the linker and the resin were not detected, making this the strategy of choice for the preparation of this kind of resin. These results have recently been corroborated during the preparation of secondary amines from BAL anchored to aminomethylpolystyrene, which involves cleavage with TFA/DCM (1:1) at 60 °C for several hours [11].



Fmoc-Rink-aminomethyl resin BAL-aminomethyl resin

Conclusions

Rink, BAL, and other similar resins are best prepared by attaching the corresponding linkers (**1**, **3**, and **4**) to an aminomethyl resin. Alternatively, linkers can be anchored to the solid support through a C–C bond [24] or other stable structures [25]. This approach avoids cleavage of linker moieties, which can contaminate the final product and remain attached to the target compounds. This phenomenon is extremely important in the preparation of compounds and linkers that require a high concentration of TFA and/or high temperatures.

Experimental Section

Materials & General Methods:

Materials and Equipment

Protected amino acids were obtained from Luxembourg Industries (Tel Aviv, Israel) and Calbiochem-Novabiochem AG (Läufelfingen, Switzerland). Fmoc-Rink linker and solid supports were supplied by Calbiochem-Novabiochem AG. DIPCDI was obtained from Fluka Chemika (Buchs, Switzerland) and HOBt from Albatross Chem Inc. (Montreal, Canada). Solvents for peptide synthesis and RP-HPLC were obtained from Scharlau (Barcelona, Spain). Trifluoroacetic acid was supplied by KaliChemie (Bad Wimpfen, Germany), 2-nitrobenzenesulphenyl chloride was obtained from Lancaster (Eastgate, England). Other chemicals were obtained from Aldrich (Milwaukee, WI) and were of the highest purity commercially available. All commercial reagents and solvents were used as received. HPLC was performed using an Alliance 2795 Waters Chromatography system with a reverse-phase Symmetry C_{18} (3.9 × 150 mm) 5 μm column with UV detection at 220 and 254 nm. Mass spectra were recorded on a Micromass ZQ Mass Spectrometer.

General Procedures

Solid-phase manipulations were carried out in polypropylene syringes fitted with a polyethylene porous disc. Solvents and soluble reagents were removed by suction. Washings between deprotection, coupling, and subsequent deprotection steps were carried out with DMF (5 × 0.5 min) and DCM (5 × 0.5 min) using 10 mL solvent/g resin each time.

Fmoc-Rink and BAL resins. The corresponding linkers (3 equiv), HOBt (3 equiv) in DMF (1–3 mL/g resin) and DIPCDI (3 equiv) were sequentially added to the MBHA-resin (0.7 mmol/g) and left to react with intermittent manual stirring for 1 h. The solvent was removed by filtration, the resin washed as indicated above, and the extent of the coupling was checked by the ninhydrin test. Incorporation of the Fmoc-Ala-OH, Fmoc-Trp-OH, and Fmoc-Tyr-OH was performed essentially as above, but after 2 h coupling.

Fmoc removal : piperidine/DMF (2:8, v/v) (2 × 10 min).

Alloc removal: Two treatments of 20 min each were performed under Ar using tetrakis(triphenylphosphine)-palladium (0.05 equiv) and phenylsilane (12 equiv) in anhydrous DCM.

Reductive amination reaction on BAL resins. The amine (5 equiv) and sodium cyanoborohydride (5 equiv) in AcOH/DMF (1:99) (8 mL/g of resin) were added to the BAL resin (1 mmol/g) (preswollen in DMF) and the mixture was stirred for 3 h at 25 °C. The resin was filtered off and washed with DMF, AcOH/DMF (1:99), DMF, DIEA/DMF (1:19), DMF, DCM, and dried.

Reductive amination reaction on Rink resins. Aldehydes (15 equiv) were condensed with Rink resin (0.7 mmol/g) in TMOF (1 mL) and the mixture was stirred under Ar for 2h at 25 °C to give the corresponding aldimines. The resin was filtered off and washed with TMOF and dry THF. $LiBH_4$ (15 equiv) in dry THF was added under Ar and the mixture was stirred for 5 h at 65 °C to give the corresponding secondary amines. The resin was filtered off and washed several times with THF, H_2O, MeOH and DCM.

Derivatization of Trp. 2-Nitrobenzenesulphenyl chloride (3 equiv) were dissolved in AcOH-DMF (8:2) and the mixture was stirred under Ar for 3 h at 25 °C.

Acylation. For acetylation, acetic anhydride (10 equiv) and DIEA (10 equiv) in DCM for 1 h at 25 °C was used. The rest of acylations were carried out with the corresponding carboxylic acids (5 equiv), DIPCDI (5 equiv) and HOBt (5 equiv) in DMF for 2 h at 25 °C.

Diethyl amine derivatives. Two treatments of 3 hours each using acetaldehyde (5 equiv) and sodium cyanoborohydride (5 equiv) in DMF at 25 °C were carried out. Then, the resin was washed with DMF (5 × 1 min), DCM (5 × 1 min), MeOH (5 × 1 min) and DCM (5 × 1 min).

Trimethylammonium derivatives. Alkylation reactions were performed using methyl iodide (10 equiv) and DIEA (10 equiv) in DCM. The mixture was stirred overnight at 25 °C.

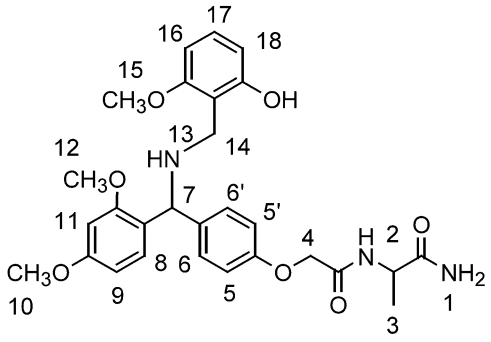
Guanidine derivatives. Triethylamine (3 equiv) and commercial available guanidium derivatives (3 equiv) were disssolved in DCM and added to the resin. The mixture was stirred overnight at 25 °C [26].

Sulphonamidation. Sulfonyl chloride (7 equiv) and triethylamine (7.3 equiv) were disssolved in DCM and added to the resin. The mixture was stirred for 18 h at 25 °C.

Cleavage of compounds from the resin was performed with TFA mixtures for 2 h at 25 °C. The solutions were filtered and evaporated to dryness.

^{1}H-NMR (500 MHz DMSO/ppm): 1.221 (d, $J = 7.0$ Hz, 3 H_3), 3.752 (s, 3 H, OMe), 3.785 (s, 3 H, OMe), 3.794 (s, 3 H, OMe), 3.949 (s, 2 H_{14}), 4.250 (q, $J = 7.0$ Hz, 1 H_2), 4.256 (s, 2 H_4), 5.743 (s, 1 H_7), 6.580 (d, $J = 9.0$ Hz, 1 $H_{6'}$), 6.782 (d, $J = 7.0$ Hz, 1 H_8), 6.798 (t, $J = 8.0$ Hz, 1 H_{17}), 6.882 (d, $J = 8.0$ Hz, 1 H_{18}), 6.885 (d, $J = 7.0$ Hz, 1 H_9), 6.957 (s, 1 H_{11}), 6.983 (d, $J = 8.0$ Hz, 1 H_6), 6.986 (d, $J = 8.0$ Hz, 1 H_{16}), 7.047 (s, 2 H_1), 7.412 (d, $J = 8.0$ Hz, 1 H_5), 7.422 (d, $J = 9.0$ Hz, 1 $H_{5'}$), 8.052 (m, 1 H, 1 H_{13}).

^{13}C-NMR (500 MHz DMSO/ppm): 18.7(C_3), 56.1(OMe), 55.1(OMe), 55.5(OMe), 37.8(C_{14}), 47.9(C_2), 66.9(C_4), 98.8($C_{6'}$), 112.5(C_{16}), 114.9(C_{11}), 119.0(C_8), 119.1(C_6), 119.1(C_{18}), 120.7(C_9), 121.9(C_{17}), 129.6(C_5), 129.6($C_{5'}$).



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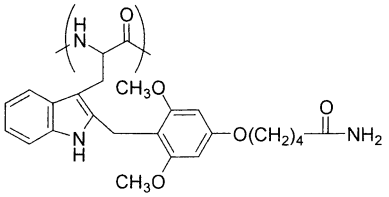

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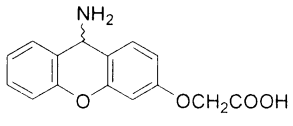

XAL linker

[21] **6a, b** (acyl and Fmoc derivatives) could only be released from the corresponding resins with 95% TFA, indicating that the presence of the free amine function makes the bond between the Trp and the linker more acid stable.
[22] It is known that primary amines (from amino acids) can not easily be released from BAL resin. Thus, *t*-butyl-protected amino acids were incorporated into a BAL resin by a reductive amination and subsequent treatment with neat TFA (6×10 min, 25 °C) removed the *t*-butyl group without cleavage of the amino acids anchored to the BAL resin: J. Alsina, T. S. Yokum, F. Albericio, G. Barany, *Tetrahedron Lett.* **2000**, *41*, 7277–7280.
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