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Aryl Hydrazides as Linkers for Solid Phase Synthesis which are Cleavable under Mild Oxidative Conditions

Christopher R. Millington, Rachel Quarrell and Gordon Lowe*

Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford, OX1 3QY, UK

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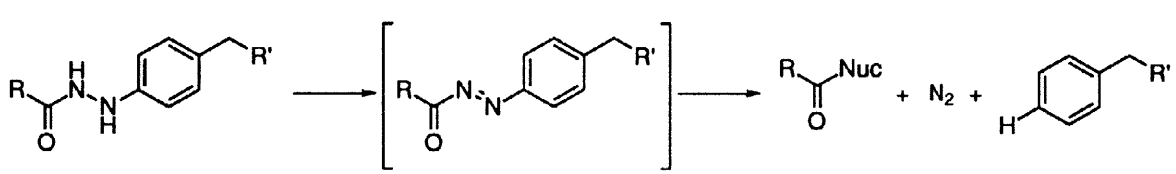
Abstract: We have developed an acid/base stable aryl hydrazide linker which is readily coupled to solid phase resins. Cleavage is specific and facile, requiring a copper (II) catalyst, base and a nucleophile to proceed. The conditions are compatible with all 20 proteinogenic amino acids and quantitative cleavage is achieved within 2 hours at 20°C to give peptides with C-terminal acid, amide or ester functionalities. Aryl hydrazides also offer scope as simple "traceless" linkers.

Keywords: linkers; oxidative cleavage; peptide synthesis; solid phase synthesis

The widespread acceptance of combinatorial chemistry as a means of generating lead compounds for biological targets[1] is placing new demands on solid phase resins and linker chemistry.[2] There is a niche for a versatile linker allowing the partial or complete release of products into solution for bioassays which is orthogonal to the conventional acid or base deprotection strategies. This paper reports a versatile linker which is readily prepared in high yield and easily attached to functionalised solid supports. The linker which is stable to acid and bases is cleaved with high specificity under mild oxidative conditions in the presence of base and a nucleophile, yielding a range of C-terminal functionalities. The linker may be used to synthesise fully or partially protected peptides for fragment coupling.

Dehydrogenation of aryl hydrazines by a variety of oxidants has long been known to produce arenes and nitrogen via a transient aryl diazene.[3] An aryl hydrazide linker is formed by coupling a carboxylic acid to an aryl hydrazine. This may be cleaved oxidatively by a range of reagents including Fehling's solution, Tollen's reagent, *N*-bromosuccinimide/pyridine and periodic acid (Scheme 1).



Scheme 1: Oxidative cleavage of an aryl hydrazide

Initial experiments were carried out in solution cleaving the model linker (**1**) with Fehling's solution[4]. Reactions proceeded completely within 2 hours at 20°C to yield only the product (**2**) and nitrobenzene (detected by GCMS). The facile cleavage of the hydrazide by this oxidant encouraged its development in preference to the other reagent combinations mentioned above (Scheme 2).

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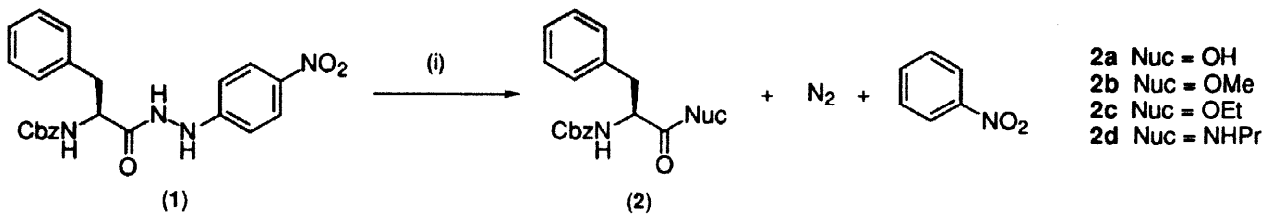


(i) HNuc (solvent) / base (1.2 equiv.) / copper (II) acetate (0.1 equiv.) / RT / 2h / >90%

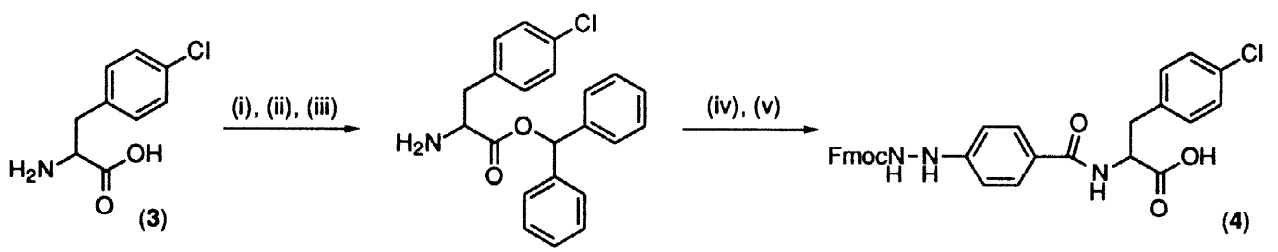
Scheme 2: Solution phase cleavage of a model hydrazide

Further solution phase investigations showed copper(II) to be required only in catalytic quantities due to rapid aerial oxidation of Cu(I) ions. This demonstrated the dual function of the nitrogen base, being required both for proton abstraction and copper ion complexation. Without a complexing agent, copper(I) oxide precipitated from solution. The system provides more rapid and efficient cleavage than other oxidative methods,[5] and allows a range of nucleophiles to be used (Table 1). Unsuccessful cleavages using ^{t}BuOH, ArOH, pentafluorophenol, *p*-cresol and benzyl alcohol revealed that the choice of HNuc is constrained by nucleophilicity and steric factors.

Table 1: Range of nucleophiles employed in solution phase cleavage of the model hydrazide (**1**)

	HNuc	Base	Yield
2a (free acid)	H_2O	DBU[6]	90%
2b (methyl ester)	MeOH	pyridine[7]	95%
2c (ethyl ester)	EtOH	DBU	93%
2d (prop-2-yl ester)	^{i}PrOH	DBU	89%
2e (*N*-propylamide)	H_2NPr	-	>95%

Having established successful conditions in solution, the system was transferred to the solid phase and *N*-Fmoc-4-hydrazino-benzoic acid (Fmoc-HBA)[8] was coupled to TentaGel resin.[9] Coupling was inefficient probably due to the poor electrophilicity of the activated Fmoc-HBA. It is thus preferable to couple Fmoc-HBA to a reference amino acid in solution, *e.g. p*-chlorophenylalanine (**3**).[10] The linker-reference (**4**) was prepared as shown in Scheme 3.



(i) Boc_2O (1 equiv.) / ^{t}BuOH / 4% NaOH (aq) / RT / 24 h
(ii) Ph_2CN_2 (1.2 equiv.) / EtOAc / RT / 12 h
(iii) PTSA (3 equiv.) / CH_3CN / RT / 3 h
(iv) Fmoc-HBA (1.2 equiv.) / DCCI (1.2 equiv.) / HOBt (1.2 equiv.) / DMF / RT / 48 h
(v) TFA (5-10 ml/mmol) / anisole (50 µl/ml of TFA) / RT / 2.5 h

Scheme 3: Synthesis of the linker-reference system

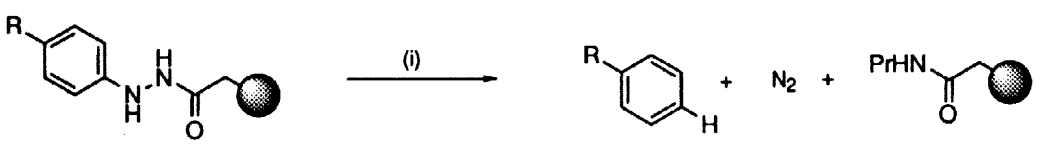
Compound (**4**) was coupled to TentaGel in excellent yields using 3 equivalents each of (**4**), DCCI and HOBt in DMF. It was shown to be completely stable to all standard solid phase peptide protocols (including Field's cleavage reagent[11]) and compatible with all 20 proteinogenic amino acids. Peptides were synthesised using Fmoc methodology.[12]

Following cleavage from the resin, copper(II) was removed from the peptidic products using either Chelex resin[13,14] or a simple DCM/dilute acid extraction.[15] Table 2 shows the range of peptides successfully synthesised and cleaved from TentaGel resin functionalised with the hydrazide-linker (**4**). All gave near quantitative cleavage within 2 hours at 20°C based on amino acid analysis. The molecular ions were detected by electrospray mass spectrometry and the crude products were between 70-90% pure by HPLC analysis at 214 nm.

Table 2: Peptides cleaved from TentaGel functionalised with the hydrazide-linker (**4**)

	Successful Cleavages (HNuc/base)	
Sequence	**Fully Protected[16]**	**Unprotected**
LVA	No protecting groups necessary	H_2O/pyridine and MeOH/pyridine
Ac-ICM	H_2NPr/-- and MeOH/pyridine	H_2NPr/-- and MeOH/pyridine
ERN	H_2NPr/-- and MeOH/pyridine	H_2NPr/-- and H_2O/DBU
KHP	H_2NPr/-- and H_2O/DBU	H_2NPr/-- and EtOH/pyridine
YWTS	H_2NPr/-- and EtOH/DBU	H_2NPr/-- and MeOH/pyridine
FQDG	H_2NPr/--	H_2NPr/--

In a broader context, the linker system offers scope for solid phase organic synthesis. Here it could be used to give the range of C-terminal functionalities described above or by reversal to give a "traceless" linker (Scheme 4). Cleavage of such a system would leave a proton at the original point of attachment to the resin, giving results comparable to Ellman's elegant but complex silicon- and germanium-based linkers.[17] The acid/base stability of the system and initial solution phase experiments have shown that the hydrazides should provide a simple and novel "traceless" linker.



(i) copper (II) acetate (0.1 equiv.) / NH_2Pr / RT / 2h

Scheme 4: A hydrazide-derived "traceless" linker

In summary, aryl hydrazides are simple, versatile linkers for use in solid phase peptide synthesis, cleaving under highly specific oxidative conditions to yield acids, amides or esters.[18] They also have potential in a reversed form as a "traceless" linker for solid phase organic synthesis.

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REFERENCES AND NOTES

1 For recent reviews see: Lowe, G., *Chem. Soc. Revs.* **1995**, 309-317, and Terret, N.K.; Gardner, M.; Gordon, D.W.; Kobylecki, R.J.; Steele, J. *Tetrahedron* **1995**, *53*, 8135.

2 For a review of current linkers refer to: Sucholeiki, I in *Combinatorial Chemistry- Synthesis and Application* ed. Wilson, S.R. and Czarnik, A.W., John Wiley and Sons, **1997**, 119-133.

3 Newbold, B.T. in *"The Chemistry of the hydrazo, azo and azoxy group"* Part 1, ed. Patai, S., John Wiley and Sons, **1975**, 542.

4 Fehling, H.; *Ann. Chem. Pharm.* **1849**, *72*, 106.

5 Semenov, A.N.; Gordeev, K., *Int. J. Pept. Prot. Res.* **1995**, *45*, 303-304.

6 **Abbreviations:** Boc, *t*-butyloxycarbonyl; Cbz, benzyloxycarbonyl; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DCCI, *N,N'*-dicyclohexylcarbodiimide; Fmoc, 9-fluorenylmethoxycarbonyl; HOBt, 1-hydroxybenzotriazole; PTSA, *p*-toluenesulphonic acid; TFA, trifluoroacetic acid; Pmc, pentamethylchromansulphonyl; Trt, trityl.

7 DBU is incompatible with copper (II) acetate/methanol causing the copper to drop out of solution as an insoluble salt.

8 Fmoc-HBA was synthesised in a single step from 4-hydrazino-benzoic acid (26 mmol) in dioxane (35 ml)/10% sodium bicarbonate (aq) (65 ml), with Fmoc-Cl (28 mmol) in dioxane (35 ml). The product was recrystallised from EtOH/EtOAc in 75% yield.

9 NovaSyn® TG resin (Novabiochem) was used and the symmetrical anhydride formed using DCCI. Three couplings were required using a five fold excess of reagent for each coupling.

10 β-alanine was initially employed but was poorly resolved in the amino acid analysis, however *p*-chlorophenylalanine had a very distinct retention time and was easily identified.

11 King, D.S.; Fields, C.G.; Fields, G.B., *Int. J. Pept. Prot. Res.* **1990**, *36*, 255.

12 For a guide to Fmoc methodology see: Atherton, E; Sheppard, R.C., *Solid Phase Synthesis: A Practical Approach*, IRL Press, Oxford, **1989**.

13 Chelex is a commercially available cation exchange resin, purchased from the Sigma Chemical Co.

14 In amide work-up, excess amine should be removed prior to use of Chelex due to competitive coordination which limits copper (II) removal. When producing esters, Chelex-promoted hydrolysis should be minimised by addition of acetic acid to neutralise the base prior to removal of copper ions.

15 For N-acylated peptides the solvents and excess base were evaporated off and the product extracted using DCM and 0.5 M HCl (aq).

16 Protecting groups used were: Pmc (Arg), Trt (Cys, His, Asn), ^{t}Bu (Glu, Ser, Thr, Tyr) and Boc (Lys).

17 Plunkett, M.J.; Ellman, J.A.; *J.Org. Chem.* **1995**, *60*, 6006-6007.

18. Fmoc-hydrazinobenzoylaminomethyl-polystyrene is available from Calbiochem-Novabiochem (see letter 2/98). The linker attached to the new high-load PEG-PS resin will be available from the same company shortly.