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LETTERS

Rapid Reaction Scanning of Solid Phase Chemistry Using Resins Incorporating Analytical Constructs

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Abstract: Two analytical constructs, based on orthogonally cleavable linkers, are reported which facilitate the mass spectral analysis of solid phase chemistry. The chemical compatibility and orthogonality of the linkers were established in a parallel reaction study using constructs prepared specifically for the purpose.

Key Words: ionisation; labelling; mass spectrometry; solid-phase synthesis

Solid-phase organic synthesis [1] is now widely accepted as the leading edge technology for assembling large compound libraries required, *inter alia*, for screening in drug discovery programs. Although resin-based chemistry offers many practical advantages over conventional solution phase methods, effective monitoring of reactions conducted on resin remains problematic, as developments in this area [2] have generally not kept pace with other advances in the field. Therefore, the refinement of reliable and cost effective methods of high throughput analysis, which have truly generic applicability, remains an important objective. The design of a dual linker, analytical construct bound to a commercially available polymeric suppport has recently been reported [3] which exploits the pivotal, earlier work of Geysen [4] and others [5] in this area (Fig.1). The construct contains an acid-cleavable group, linker 1, allowing the release of reaction substrates in the conventional manner, and an orthogonal, photolabile carbamate, linker 2, which provides for chemoselective cleavage in the 'analytical mode'. The latter process releases an amine-containing fragment which has enhanced electrospray ionisation properties and is isotopically labelled, uniquely facilitating the identification of relevant signals in the mass spectrum.

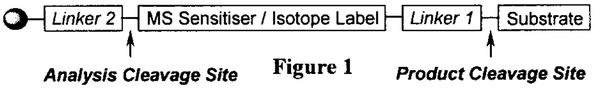

Figure 1

Herein, we report the preparation of two new analytical constructs, based on alternative amine-releasing linkers, thereby extending the range of chemistry to which this method can be applied. The suitability of the Dde [6] and the 2-nitrophenyl sulfonamide linkers [7] as the amine-releasing components, linker 2, was first established in a separate reaction compatibility scan by incorporating

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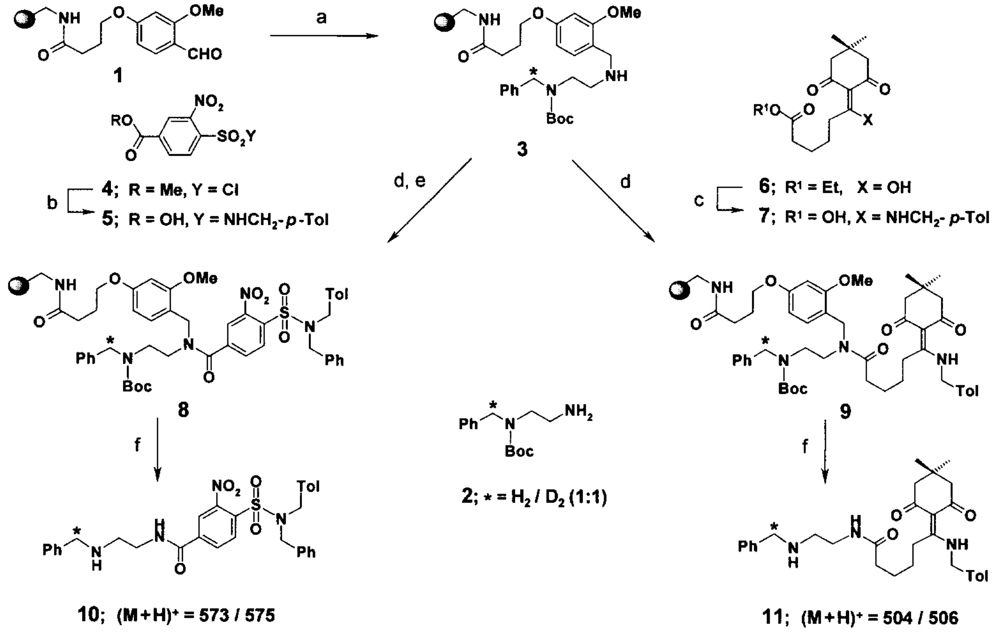
them 'downstream' of the analytical fragment as linker 1 (Scheme 1). Reductive amination of the aldehyde linker **1**, (on aminomethyl ArgoGel™ resin) with *N*-benzyl-*N*-Boc-ethylenediamine (50% doubly labelled with deuterium) **2** gave the immobilised benzylamine **3**. The Dde derivatised acid **7**, readily prepared [6] from the 2-acyldimedone **6**, was coupled to the amino resin under standard conditions to provide 'scanning construct' **9**. The corresponding sulfonamide containing construct **8** was similarly prepared from the benzoic acid derivative **5**, obtained in two steps from the readily available [7] sulfonyl chloride **4**. Acidolysis of the resins **8** and **9** released the capped analytical fragments **10** and **11**, respectively, which showed strong signals for the expected ion pairs in the mass spectra.



Scheme 1: a) 2, Na(OAc)$_3$BH, 1% AcOH in DMF, RT, 16 h, >95%; b) i. p-TolCH$_2$NH$_2$, Et$_3$N, CH$_2$Cl$_2$, 16 h, 0 °C to RT, 86%, ii. NaOH (aq), MeOH, RT, 20 min, 90%; c) i. NaOH (aq), THF, RT, 5 h, 85%; ii. p-TolCH$_2$NH$_2$, DIPEA, CH$_2$Cl$_2$, RT, 7 h, 97%; d) Acid 5 or 7, PyBOP, HOBt, DIPEA, DMF, CH$_2$Cl$_2$, 16 h, quant.; e) BnOH, PPh$_3$, DEAD, THF, 16 h, RT, quant.; f) TFA, CH$_2$Cl$_2$, (1:1), RT, 30 min.

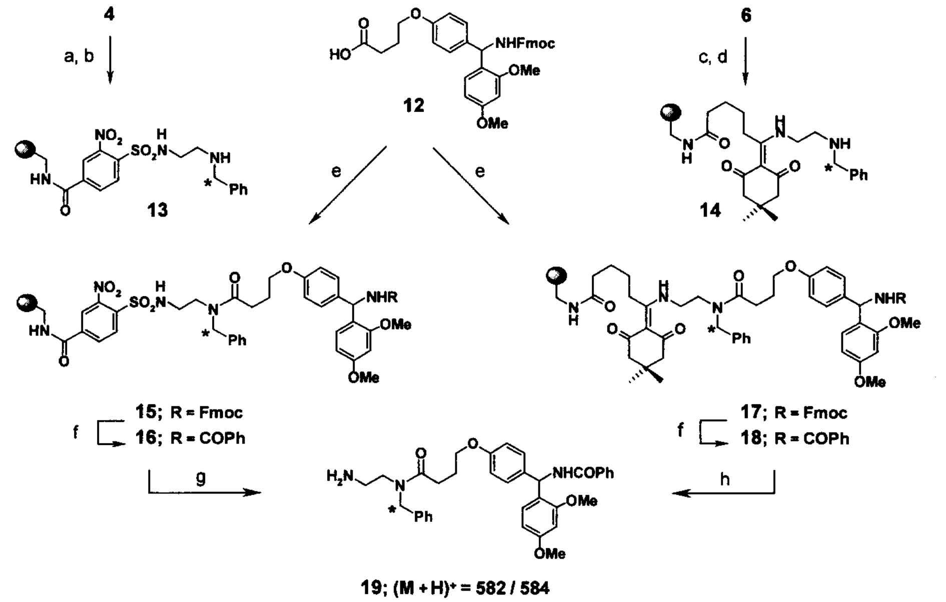
Incubation of bead aliquots to a range of commonly used reagents was performed in parallel, followed by subsequent cleavage and analysis by LCMS (Table). Reagents which are used to cleave the sulfonamide (Entry 1) and the Dde group (Entry 3) were included as positive controls. As expected, the Dde group was attacked by sodium borohydride, (Entry 13) whilst tin(II) chloride (Entry 15) reduced the aromatic nitro group in the sulfonamide. Surprisingly, the sulfonamide linker proved to be quite labile under strongly basic conditions (Entries 6, and 20) and, to a lesser degree, towards fluoride anion (Entry 21). The results from this study reveal that both linkers are stable to a wide range of reagents and demonstrate the power of the construct in solid-phase reaction analysis. A more detailed study, to define alternative cleavage conditions for the removal of Fukuyama's sulfonamide protective group [8] will be reported in due course. Exposure of resins **8** or **9** to *m*-CPBA (Entry 16) failed to provide any

Table: Reaction Scans for the Sulfonamide and Dde Linkers in Constructs the **8** and **9**

Entry	Reaction Conditions[a]	10	11	Entry	Reaction Conditions[a]	10	11
1	Mercaptoethanol, DBU, DMF	✓	×	**12**	$PhCH_2Br$, Cs_2CO_3, DMF	✓	✓
2	20% Piperidine, DMF	✓	✓	**13**	$NaBH_4$, THF	×	✓
3	2% Hydrazine, DMF	×	✓	**14**	PhCHO, $Na(OAc)_3BH$, 1% AcOH, DMF	✓	✓
4	Phenyltetrazole, KOtBu	✓	✓	**15**	$SnCl_2.2H_2O$, DMF	✓	×
5	10% MeOH, THF	✓	✓	**16**	*m*-CPBA, CH_2Cl_2	×	×
6	NaOMe, MeOH, THF	✓	×	**17**	H_2O_2 (10%), 30 vols, THF	✓	✓
7	$Ph_3P{=}CH(C{=}O)Me$, THF	✓	✓	**18**	PPh_3, $PhCH_2OH$, DEAD, CH_2Cl_2	✓	✓
8	PyBOP, HOBt, DIPEA, $PhCO_2H$, DMF	✓	✓	**19**	$Pd(PPh_3)_4$, HOBt, THF	✓	✓
9	Ac_2O, DIPEA, CH_2Cl_2	✓	✓	**20**	KOTMS, THF	✓	×
10	PhCOCl, pyridine, CH_2Cl_2	✓	✓	**21**	TBAF (1M), THF	✓	×
11	$MeSO_2Cl$, DIPEA, CH_2Cl_2	✓	✓	**22**[b]	hv, DMSO	✓	✓

a) Resin aliquots, ~20 mg, were incubated with reagents (5 equiv.) in a HiTop Block (*ex* Whatman, catalogue no.: PolyWhat 004) at RT for 3 h and then cleaved with 50% TFA, CH_2Cl_2. LCMS analyses were performed on a Hewlett Packard HP 1050 instrument (diode array detection) and a Micromass Platform I (8084) mass spectrometer using electrospray ionisation in +ve mode; ✓ ≥85% **10** or **11** by peak area; **b)** Conducted as a separate experiment: 20 mg of resin in 0.5 ml solvent was irradiated at 365 nM, for 3 h.

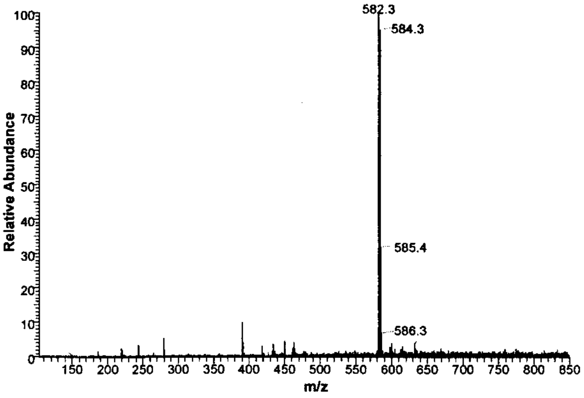
construct-derived signals in the mass spectrum, presumably due to the sensitivity of the dialkyl resorcinol nucleus, present in the aldehyde linker, towards oxidation. In spite of these limitations the Dde and sulfonamide groups possess a high degree of orthogonality with conventional, acid-cleavable resin linkers and this was exploited by incorporating them, as linker 2, in two new analytical constructs **15** and **17** for use in solid-phase reaction monitoring (Scheme 2).



Scheme 2: **a)** i. **2**, Et_3N, CH_2Cl_2, RT, 2 h, 79%; ii. NaOH (aq), MeOH, RT, 1 h, 88%; **b)** i. Novabiochem AM resin HL, PyBOP, HOBt, DIPEA, DMF, CH_2Cl_2, 16 h, quant.; ii. TFA, Phenol, CH_2Cl_2, RT, 10 min, >95%; **c)** i. NaOH (aq), THF, RT, 3 h, 95%; ii. **2**, DIPEA, CH_2Cl_2, RT, 20 h, 95%; **d)** i. Aminomethyl ArgoGel™, PyBOP, HOBt, DIPEA, DMF, CH_2Cl_2, 16 h, quant.; ii. TFA, Phenol, CH_2Cl_2, RT, 10 min, >95%; **e)** Amine **13** or **14**, PyBOP, HOBt, DIPEA, DMF, CH_2Cl_2, 16 h, quant.; **f)** i. 20% Piperidine, DMF, RT; ii. $PhCO_2H$, PyBOP, HOBt, DIPEA, DMF, CH_2Cl_2, 16 h, quant.; **g)** $HOCH_2CH_2SH$, DBU, MeCN, RT, 1 h; **h)** 2% $N_2H_4.H_2O$, DMF, RT, 30 min.

Reaction of the diamine **2** with the sulfonyl chloride **4**, followed by ester saponification, coupling to high load aminomethylated polystyrene resin, and *N*-Boc deprotection gave the sulfonamide construct precursor **13**. The corresponding Dde containing construct intermediate **14** was assembled in an analoguous fashion from the acyl dimedone **6** on aminomethyl ArgoGel™ resin. The benzylamines **13** and **14** are versatile intermediates, readily permitting the generation of a family of constructs by subsequent coupling to any suitable linker of choice. In our case, the amines were coupled to the Rink amide derivatised acid **12** providing the Dde and sulfonamide containing constructs **15** and **17**. Removal of the Fmoc protective group in each case and acylation with benzoic acid gave the 'capped' constructs **16** and **18**, which were subjected to the appropriate cleavage process in order to demonstrate their utility. The reductive cleavage of the sulfonamide **16** with mercaptoethanol as well as treatment of the Dde construct **18** with hydrazine provided the isotopically labelled primary amine **19**, which was readily visible as a strong 'doublet' signal [m/z; 582 and 584] in the mass spectrum (Fig. 2).

Figure 2



In summary, resin constructs, such as those disclosed above, have considerable potential in many areas of solid phase synthesis where reaction monitoring is frequently still difficult. Using two constructs, the chemical stability and reactivity profile of the sulfonamide and Dde linkers were readily established in a rapid, parallel, solid–phase study demonstrating the unique power of the methodology as an analytical tool. The use of these and other analytical constructs in assessing resin linker stabilities, the orthogonality of protective groups and reaction scanning and optimisation is currently underway and will be reported in due course.

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