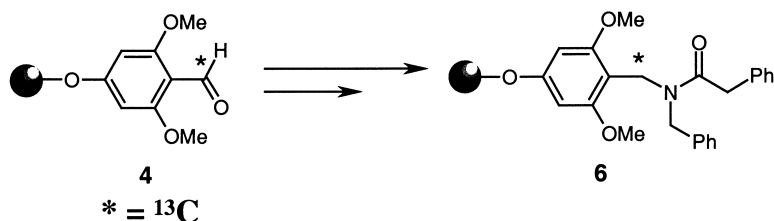Article

Development and Application of a Carbonyl-C-Enriched Backbone Amide Linker for Solid-Phase Reaction Monitoring

Craig Jamieson, Miles S. Congreve, Peter R. Hewitt, Jan J. Scicinski, and Steven V. Ley

More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Development and Application of a Carbonyl-^{13}C-Enriched Backbone Amide Linker for Solid-Phase Reaction Monitoring

Craig Jamieson,[†] Miles S. Congreve,*,[‡] Peter R. Hewitt,[†] Jan J. Scicinski,[‡] and Steven V. Ley[†]

Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, U.K., and GlaxoSmithKline R&D, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2, 1EW, U.K.

The synthesis and application of a carbonyl-^{13}C backbone amide linker are described. The labeled unit is conveniently mixed with commercial resins, providing a rapid means of monitoring chemistry performed with this linker on solid support using conventional ^{13}C NMR methods.
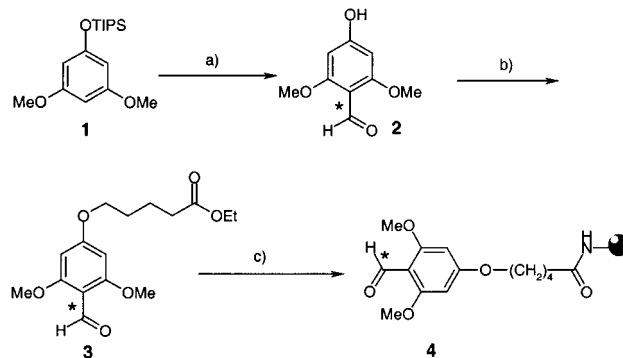
The application of cogent analytical methods is pivotal to the critical appraisal of chemistry carried out on a solid support.[1] Our laboratories and others have been active in developing approaches to solid-phase reaction monitoring, including the application of analytical construct techniques[2] and magic angle spinning (MAS) NMR,[3] infrared,[4] mass spectrometric,[5] and Raman[6] methods. In addition, the use of heteronuclear monitoring, including ^{31}P,[7] ^{19}F,[8] and ^{15}N NMR methods,[9] and the use of gel-phase ^{13}C NMR[10] have received widespread attention.

The deployment of ^{13}C-enriched species in monitoring solid-phase chemistry has also been reported.[11] These systems rely on the use of isotopically enriched *substrates,* enabling rapid acquisition of NMR data using only small amounts of material. However, a ^{13}C-labeled *linker* system would be of greater general utility than the existing substrate-labeled methods because it would obviate the need to prepare a range of labeled substrates. In addition, a ^{13}C-enriched system would have no effect on the chemical properties of the linker and, unlike ^{19}F-labeled moieties, would not electronically bias the system with consequent effects on reactivity.[8h]

The backbone amide linker (BAL), developed by Albericio and Barany,[12] has been extensively used in the preparation of secondary carboxamides and in C-terminal modification of peptides. We envisaged that installation of a ^{13}C label in this unit would enable a rapid and nondestructive means of assessing the extent of the steps of reductive amination and acylation before acid-mediated release of substrates from resin. Also, this system allows the experimentalist to determine the extent of each of these reactions, thus ensuring complete loading of the linker and hence maximizing yield. Evidently, an NMR-based method that enables one to determine the extent of reaction would be superior to IR methods or functional group spot tests,[13] which may often give ambiguous results.

From consideration of ^{13}C NMR predictions,[14] placement of a ^{13}C label at the aldehyde functional group would be

---

[†] Department of Chemistry, University of Cambridge.
[‡] GlaxoSmithKline R&D, Department of Chemistry, University of Cambridge.

**Scheme 1.** Preparation of the ^{13}C-Labeled BAL[a]

[a] Reagents: (a) *n*-BuLi, *N,N*-dimethylformamide-*carbonyl*-^{13}C, THF, room temp, 79%; (b) ethyl-5-bromovalerate, 2-*tert*-butylimino-2-diethyl-amino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine on polystyrene, 10% (v/v) MeCN/DMF, room temp, 66%; (c) (i) NaOH, THF, room temp; (ii) ArgoGel-NH₂, PyBOP, DIEA, CH₂Cl₂/DMF, room temp.

most diagnostic in terms of changes in chemical shift in the reductive amination/acylation sequence. Therefore, 4-*[13]C*-formyl-3,5-dimethoxyphenol was prepared by lithiation of the triisopropylsilyl derivative of 3,5-dimethoxyphenol (**1**) followed by quenching with *N,N*-dimethylformamide-*carbonyl*-[13]C (Scheme 1).[15] Introduction of a valeryl spacer and loading onto amino-functionalized ArgoGel was achieved by adaptation of literature protocols.[12b] The extent of loading was estimated by derivatization of the aldehyde resin (**4**) with 9-fluorenylmethyl carbazate[16] followed by spectrophotometric measurement of a suspension of the resin in a solution of 20% (w/v) piperidine/DMF[17] and was found to be in the range 0.25–0.32 mmol/g.

With the aldehyde resin (**4**) in hand, a representative synthetic sequence was examined (Scheme 2). Reductive amination of (**4**) with benzylamine in the presence of tetra-*N*-butylammonium borohydride, followed by PyBOP-mediated acylation with phenyl acetic acid, furnished the resin-bound carboxamide (**6**). The synthesis was monitored at each stage using gel-phase ^{13}C NMR together with ^{1}H MAS NMR in order to confirm the identity of each species. Additionally, initial studies indicated that the labeled resin could readily be mixed with conventional resins in a 1:1 ratio with no

Scheme 2. Synthesis of Resin-Bound Carboxamide (**4**)[a]
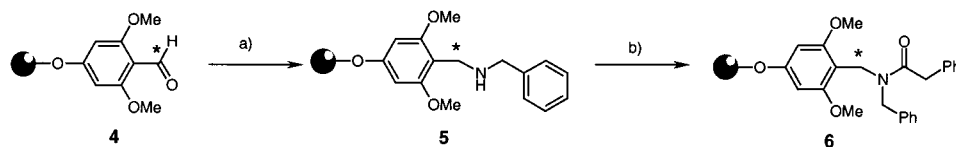
[a] Reagents: (a) (i) benzylamine, Bu₄NBH₄, AcOH, NMP, room temp, (ii) 50% (v/v) TFA/CH₂Cl₂; (b) phenyl acetic acid, PyBOP, HOBt, DIEA, DMF, room temp.
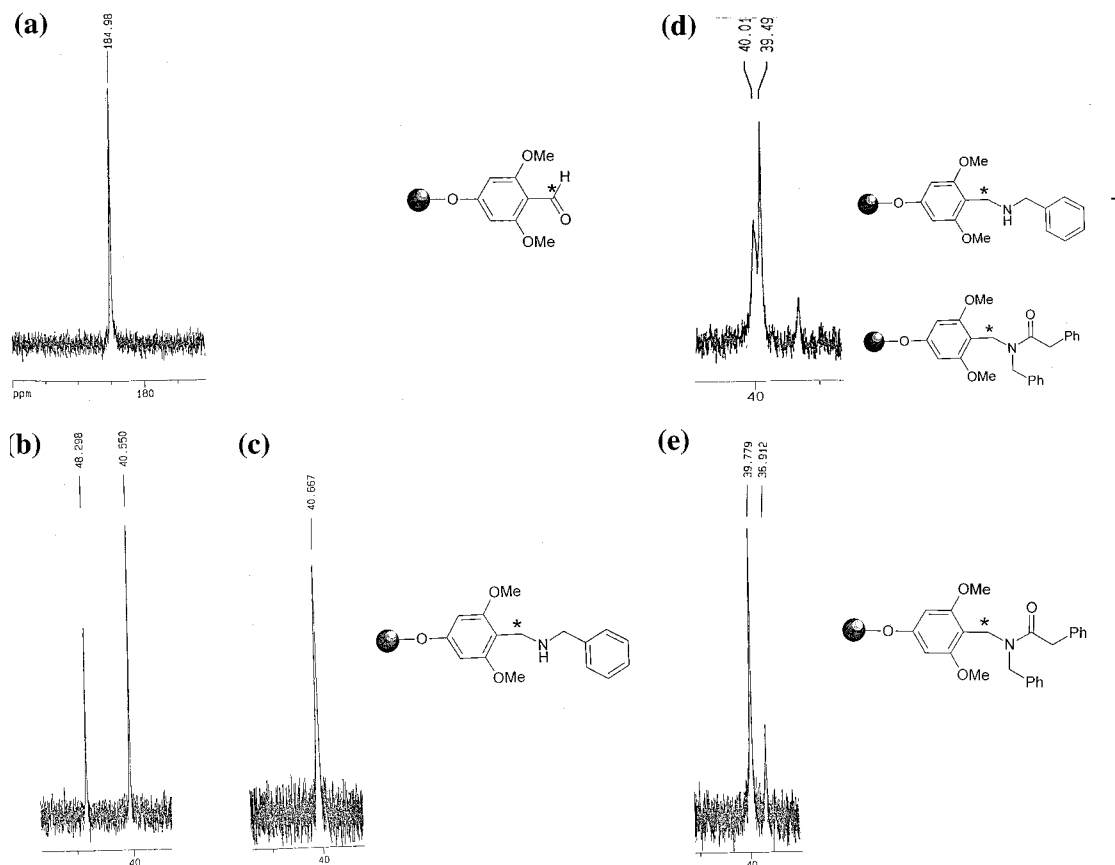

Figure 1. (a) BAL−aldehyde resin (**1**); (b) resin **5** prior to TFA treatment; (c) resin **5** following TFA treatment; (d) mixture (1:1) of resins **5** and **6**; (e) resin-bound carboxamide (**6**).

detriment to the signal-to-noise ratio and without recourse to protracted acquisition times.

Figure 1 shows the gel-phase ¹³C NMR spectra after each stage of the synthetic sequence. Each spectrum was conveniently obtained in less than 10 min using standard tubes in a hands-on instrument, providing qualitative data on the course of each reaction. This result establishes the principle of using reduced amounts of the ¹³C label. Clearly, lower levels of ¹³C reporter could therefore be employed in conjunction with longer acquisition times. Upon reductive amination, a large change in chemical shift (185−41 ppm) was observed. An additional component (48 ppm) was observed and was ascribed to a boronate adduct of the amine resin.[18] This adduct may be hydrolyzed by treatment of the resin with a mixture of TFA/CH₂Cl₂ (1:1) to yield the amine (**5**) as a single component (Figure 1c). Other intermediates including imines may also be detected in this way. A 1:1 mixture of resins **5** and **6** gave rise to three distinct peaks in the ¹³C NMR, corresponding to the amine-containing resin (40 ppm) and a rotameric mixture of acylated products (39.8 and 36.9 ppm), this being confirmed by ¹H MAS NMR. Hence, when this approach is used, it is possible to determine

the extent of both the critical reductive amination and acylation steps. Acidolytic cleavage from the resin furnished the desired carboxamide in a spectroscopically pure form. Using the same labeled linker system, we have also demonstrated that it is possible to monitor this synthetic sequence when applied to polystyrene-based supports, where MAS NMR is often difficult to obtain.

In summary, we have demonstrated how a readily prepared ¹³C label can be economically employed to give rapid, high-quality information in this widely used reaction sequence using standard NMR methods.

Experimental Details for Preparation of Resin 4

4-¹³C-Formyl-3,5-dimethoxyphenol (2). 2 was prepared exactly according to the procedure of Landi and Ramig[15] using carbonyl-¹³C DMF obtained from Sigma-Aldrich, Ltd.

Ethyl 5-(4-¹³C-Formyl-3,5-dimethoxyphenoxy) Valerate (3). To a solution of crude **2** (100 mg, 0.55 mmol) in DMF/acetonitrile (10% v/v, 4 mL) was added PS−BEMP (Fluka, 2.2 mmol/g, 742 mg, 1.65 mmol) followed by ethyl-5-bromovalerate (87 µL, 0.55 mmol). After agitation for 40 h the reaction was filtered and the resin was washed with DMF

(2 × 2 mL) and acetonitrile (3 × 5 mL). The combined filtrates were dried and purified by Biotage chromatography (2:1 hexane/ethyl acetate) to give the *title compound* as a beige solid (112 mg, 66%). ^{1}H NMR (400 MHz, CDCl$_3$), δ_H: 10.3 ppm (d, 1H, $J = 86.4$ Hz); 6.05 ppm (s, 2H); 4.15 ppm (q, 2H); 4.05 ppm (t, 2H); 3.87 ppm (s, 6H); 2.38 ppm (t, 2H); 1.85 ppm (m, 4H); 1.25 ppm (t, 3H). ^{13}C NMR (63 MHz, toluene-d_8): $\delta_c = 184.86$ ppm. ESI-MS: MH$^+ = 312$.

Resin-Bound 5-(4-^{13}C-Formyl-3,5-dimethoxyphenoxy) Valeric Acid on ArgoGel-NH$_2$ (4). To a solution of **3** (100 mg, 0.29 mmol) in methanol (2 mL) was added aqueous NaOH solution (4 N, 1 mL), and the mixture was stirred for 1 h. The reaction mixture was diluted with water (10 mL), acidified, and extracted with ethyl acetate (3 × 10 mL). The combined extracts were dried (MgSO$_4$), filtered, and evaporated under reduced pressure to yield a beige solid (88 mg). To a solution of the solid in DMF (0.25 mL) was added DIEA (93 μL, 0.54 mmol) and PyBOP (135 mg, 0.27 mmol). The resulting solution was added to aminomethyl ArgoGel (Argonaut, 0.40 mmol/g, 338 mg, 0.14 mmol), which was preswollen in CH$_2$Cl$_2$ (1 mL). The whole was shaken overnight before being washed with DMF (3 × 20 mL), CH$_2$-Cl$_2$ (3 × 20 mL), and ether (3 × 10 mL) to give the title resin (391 mg).

References and Notes

(1) Schwartz, M. *Analytical Methods in Combinatorial Chemistry*; Marcel Dekker: New York, 2000.

(2) (a) Williams, G. M.; Carr, R. A. E.; Congreve, M. S.; Kay, C.; McKeown, S. C.; Murray, P. J.; Scicinski, J. J.; Watson, S. P. *Angew. Chem., Int. Ed.* **2000**, *39*, 3293. (b) McKeown, S. C.; Watson, S. P.; Carr, R. A. E.; Marshall, P. *Tetrahedron Lett.* **1999**, *40*, 2407. (c) Murray, P. J.; Kay, C.; Scicinski, J. J.; McKeown, S. C.; Watson, S. P.; Carr, R. A. E. *Tetrahedron Lett.* **1999**, *40*, 5609.

(3) (a) Shea, K. J.; Sasaki, D. Y. *J. Am. Chem. Soc.* **1991**, *113*, 4109. (b) Fitch, W. L.; Detre, G.; Holmes, C. P.; Shoolery, J. N.; Keifer, P. A. *J. Org. Chem.* **1994**, *59*, 7955. (c) Anderson, R. C.; Stokes, J. P.; Shapiro, M. J. *Tetrahedron Lett.* **1995**, *36*, 5311. (d) Pursch, M.; Schlotterbeck, G.; Tseng, L. H.; Albert, K. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2867. (e) Keifer, P. A. *J. Org. Chem.* **1996**, *61*, 1558. (f) Wehler, T.; Westman, J. *Tetrahedron Lett.* **1996**, *37*, 4771. (g) Meissner, A.; Bloch, P.; Humpfer, E.; Spraulo, M.; Sorensen, O. W. *J. Am. Chem. Soc.* **1997**, *119*, 1787. (h) Ghalluin, C.; Boutillon, C.; Tartar, A.; Lippens, G. *J. Am. Chem. Soc.* **1997**, *119*, 10494. (i) Shapiro, M. J.; Chin, J.; Marti, R. E.; Jaroninski, M. A. *Tetrahedron Lett.* **1997**, *38*, 1333. (j) Chin, J.; Fell, B.; Pochapsky, S.; Shapiro, M. J.; Wareing, J. R. *J. Org. Chem.* **1998**, *63*, 1309. (k) Grice, P.; Leach, A. G.; Ley, S. V.; Massi, A.; Mynett, D. M. *J. Comb. Chem.* **2000**, *2*, 491−495.

(4) (a) Frechet, J. M. J.; Schuerch, C. *J. Am. Chem. Soc.* **1971**, *93*, 492. (b) Yan, B.; Kumaravel, G.; Anjaria, H.; Wu, A. Y.; Petter, R. C.; Jewell, C. F.; Wareing, J. R. *J. Org. Chem.* **1995**, *60*, 5736. (c) Yan, B.; Kumaravel, G. *Tetrahedron* **1996**, *52*, 843. (d) Chan, T. Y.; Chen, R.; Sofia, M. J.; Smith, B. C.; Glennon, D. *Tetrahedron Lett.* **1997**, *38*, 2821.

(5) (a) Drouot, C.; Enjalbal, C.; Fulcrand, P.; Martinez, J.; Aubagnac, J. L.; Combarieu, R.; De Puydt, Y. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 1509. (b) Egner, B. J.; Bradley, M. *Tetrahedron* **1997**, *53*, 14021. (c) Aubagnac, J. L.; Enjalbal, C.; Subra, G.; Bray, A. M.; Combarieu, R.; Martinez, J. *J. Mass. Spectrom.* **1998**, *33*, 1094. (d) Enjalbal, C.; Maux, D.; Subra, G.; Martinez, J.; Combarieu, R.; Aubagnac, J. L. *Tetrahedron Lett.* **1999**, *40*, 6217. (e) Heinze, J.; Winterhaller, U.; Jannack, T. *Chem.−Eur. J.* **2000**, *6*, 4203.

(6) Pivonka, D. E.; Sparks, R. B. *Appl. Spectrosc.* **2000**, *55*, 1584.

(7) Johnson, C. R.; Zhang, B. *Tetrahedron Lett.* **1995**, *36*, 9253.

(8) (a) Manatt, S. L.; Amsden, C. F.; Bettison, C. A.; Frazer, W. T.; Gudman, J. T.; Lenk, B. E.; Lubetich, J. F.; McNelly, E. A.; Smith, S. C.; Templeton, D. J.; Pinnell, R. P. *Tetrahedron Lett.* **1980**, *21*, 1397. (b) Albericio, F.; Pons, M.; Pedroso, E.; Giralt, E. *J. Org. Chem.* **1989**, *54*, 360. (c) Shapiro, M. J.; Kumaravel, G.; Petter, R. C.; Beveridge, R. *Tetrahedron Lett.* **1996**, *37*, 4671. (d) Svensson, A.; Fex, T.; Kihlberg, J. *Tetrahedron Lett.* **1996**, *37*, 7649. (e) Svensson, A.; Berquest, K. E.; Fex, T.; Kihlberg, J. *Tetrahedron Lett.* **1998**, *39*, 7193. (f) Stones, D.; Miller, D. J.; Beaton, M. W.; Rutherford, T. J.; Ganii, D. *Tetrahedron Lett.* **1998**, *37*, 7649. (g) Drew, M.; Orton, E.; Krolikowski, P.; Salvino, J. M.; Kumar, N. V. A. *J. Comb. Chem.* **2000**, *2*, 8. (h) Svensson, A.; Fex, T.; Kihlberg, J. *J. Comb. Chem.* **2000**, *2*, 736.

(9) Swayze, E. E. *Tetrahedron Lett.* **1997**, *38*, 8643.

(10) (a) Frechet, J. M. J. *Tetrahedron* **1981**, *37*, 663. (b) Giralt, E.; Rizo, J.; Pedroso, E. *Tetrahedron* **1984**, *40*, 4141. (c) Barany, G.; Kneib-Cordonier, N.; Mullen, D. G. *Int. J. Pept. Protein Res.* **1987**, *30*, 705. Lorge, F.; Wagner, A.; Mioskowski, C. *J. Comb. Chem.* **1999**, *1*, 25.

(11) (a) Look, G. C.; Holmes, C. P.; Chinn, J. P.; Gallop, M. A. *J. Org. Chem.* **1994**, *59*, 7588. (b) Sarkar, S. K.; Garigipati, R. S.; Keifer, P. A. *J. Am. Chem. Soc.* **1996**, *118*, 2305. (c) Kanemitsu, T.; Kanie, O.; Wong, C. H. *Angew. Chem., Int. Ed.* **1998**, *37*, 3415.

(12) (a) Albericio, F.; Barany, G. *Int. J. Pept. Protein Res.* **1987**, *30*, 206. (b) Albericio, F.; Kneib-Cordonier, N.; Biancalana, S.; Gera, L.; Masada, R. I.; Hudson, D.; Barany, G. *J. Org. Chem.* **1990**, *55*, 3730. (c) Sharma, S. K.; Songster, M. F.; Colpitts, T. L.; Hegyes, P.; Barany, G.; Castellino, F. *J. Org. Chem.* **1993**, *58*, 4993. (d) Songster, M. F.; Vagner, J.; Barany, G. *Lett. Pept. Sci.* **1996**, *2*, 265. (e) Jensen, K. J.; Alsina, J.; Songster, M. F.; Vagner, J.; Albericio, F.; Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 5441.

(13) For determination of hydroxyl see: Attardi, M. E.; Falchi, A.; Taddei, M. *Tetrahedron Lett.* **2000**, *41*, 7395. For thiols see: Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82*, 70. For amines see: Kaiser, E.; Colescott, R. L.; Bossinger. C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.

(14) Calculation of ^{13}C chemical shift was performed using the ACD/CNMR database, Advanced Chemistry Development, Inc. 1998.

(15) Landi, J. J.; Ramig, K. *Synth. Comm.* **1991**, *21*, 167−171.

(16) Zhang, R. E.; Cao, Y. L.; Hearn, M. W. *Anal. Biochem.* **1991**, *195*, 160. 9-Fluorenylmethyl carbazate is commercially available form Fluka.

(17) Atherton, E.; Sheppard, R. C In *Solid Phase Peptide Synthesis: A Practical Approach*; IRL Press: Oxford, 1989.

(18) Houghten and co-workers have reported resin-bound amine−boronate complexes: Ostresh, J. M.; Schoner, C. C.; Hamashin, V. T.; Nefzi, A.; Meyer, J. P.; Houghten, R. A. *J. Org. Chem.* **1998**, *63*, 8622.