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An Improved Aldehyde Linker for the Solid Phase Synthesis of Hindered Amides

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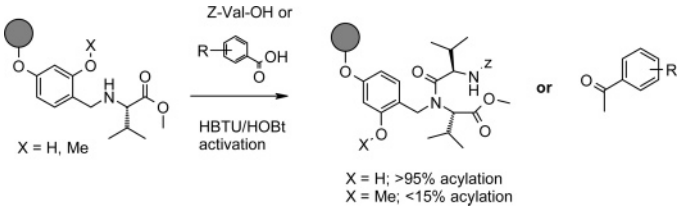
Received August 18, 2005



A novel aldehyde dual-linker system has been developed for the solid phase synthesis of sterically hindered amides. The linker [5-(4-formyl-3-hydroxyphenoxy)pentanoic acid] exploits an intramolecular oxygen−nitrogen acyl transfer mechanism to prepare compounds that are unattainable with current commercially available linkers. A dual linker system, exploiting the hyper-acid labile Sieber amide linker as part of the construct, enabled the initial reductive alkylation reactions of hindered amines and their subsequent acylation with a range of carboxylic acids with varying stereoelectronic properties to be monitored. Simple acylation conditions (HBTU/HOBt/NMM) sufficed to provide near quantitative reaction of test acids with support-bound hindered amines, reaction conditions which failed when commercial linkers were used.

Introduction

In recent years there has been an explosion of interest in the research and development of novel linkers for polymer supported synthesis[1] and several reviews have been published covering this area.[2] Originally, linkers were designed almost exclusively to aid peptide and nucleotide synthesis. However, the advent of combinatorial chemistry techniques has led to a demand for a wider range of linkers for solid-phase synthesis. This is because the original linkers have one major drawback: most leave a functional group attached to the cleaved molecule that was the attachment point for the linker (e.g., a carboxylic acid, an amide, or an alcohol). This is not always desirable for combinatorial library synthesis.

In our current research, amino acids are used as starting points for the design and synthesis of potential enzyme inhibitors. We required a simple solid-phase method that would enable an amino acid to be functionalized at both its N- and C-termini with minimal epimerization. We considered the most facile way to achieve this was through monovalent attachment of the conserved nitrogen to a linker, thus allowing simple synthetic manipulation of both termini prior to resin cleavage. Using this approach, libraries of compounds could be prepared that would not contain an unwanted functional group derived from the amino acid attachment point to the linker.

Such linkers have been developed to enable the solid phase synthesis of C-terminally modified peptides[3] and although the polymer support may vary, there are essentially only four types of this linker commercially available. (Figure 1).

These have been utilized in the literature to facilitate the synthesis of a variety of different compounds[4] with some specific examples including benzimidazoles[5] and alkoxyketones.[6]

Although all of these linkers have been shown to be effective, they all have limited use for the formation of amide bonds. This

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(1) (a) Yan, L. Z.; Mayer, J. P. *J. Org. Chem.* **2003**, *68*, 1161. (b) Golisdlae, A.; Herforth, C.; Quirijen. L.; Maes, L.; Link, A. *Bioorg. Med. Chem.* **2002**, *10*, 159. (c) Chhabra, S. R.; Parekh, H.; Khan, A. N.; Bycroft, B. W.; Kellam, B. *Tetrahedron Lett.* **2001**, *42*, 2189. (d) Andrews, S. P.; Ladlow, M. *J. Org. Chem.* **2003**, *68*, 5525. (e) Portal, C.; Launay, D.; Merritt, A. *J. Comb. Chem.* **2005**, *7*, 554.

(2) (a) Blaney, P.; Grigg, R.; Sridharan, V. *Chem. Rev.* **2002**, *102*, 2607. (b) Guillier, F.; Orain, D.; Bradley, M. *Chem. Rev.* **2000**, *100*, 2091.

(3) Jenson, K. J.; Alsina, J.; Songster, M. F.; Vagner, J.; Albericio, F.; Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 5441.

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Published on Web 01/27/2006

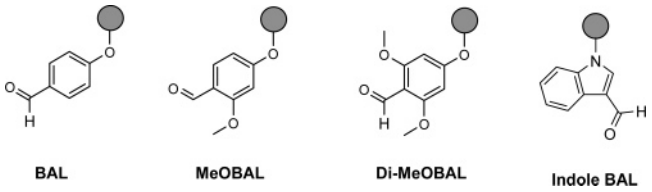
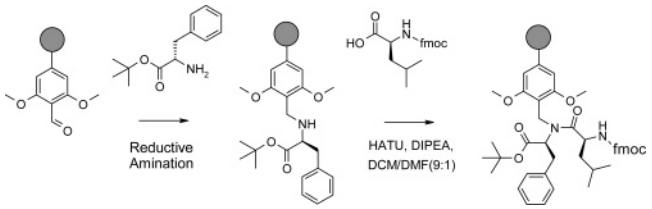
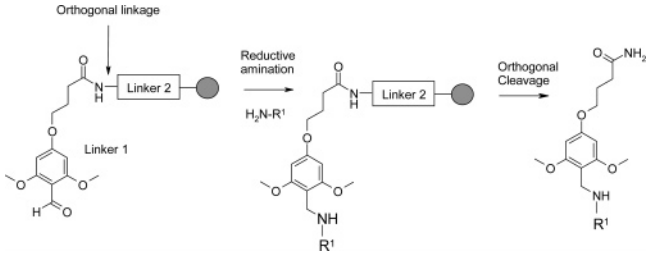


FIGURE 1. Commercially available backbone amide linkers (BAL).

SCHEME 1



SCHEME 2. "Double Linker" Strategy



problem is illustrated in Scheme 1: reductive alkylation proceeds smoothly (e.g., with phenylalanine) but subsequent acylation is achieved only when using highly activated coupling reagents.[3] Even so, acylation (e.g., with the relatively unhindered leucine) is still not quantitative.[3]

Results and Discussion

Our research initially focused on defining the limitations of one of the commercially available aldehyde linkers with respect to the formation of amide bonds. To aid this investigation, a solid phase "double linker" strategy was developed to allow the rapid analysis of solid supported postreaction products. The principle of this technique is shown in (Scheme 2).

This strategy enables the progress of reactions (e.g., reductive alkylation and acylation) to be monitored via simple cleavage and HPLC analysis of the resulting product(s). Since the aldehyde linker (**1**) is UV active, both starting material and products can be readily detected. The commercially available aldehyde linker chosen for the initial studies was 4-(4-formyl-3-methoxyphenoxy)butanoic acid (MeOBAL) (**1**) (Figure 2).

The aldehyde handle was first attached to polystyrene resin functionalized with the hyper-acid labile Sieber amide linker (Figure 3). Reductive alkylation and acylation were then performed as described below. The newly synthesized molecule

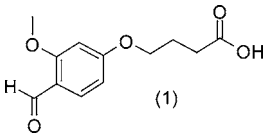


FIGURE 2. 4-(4-Formyl-3-methoxyphenoxy)butanoic acid (MeOBAL) (**1**).

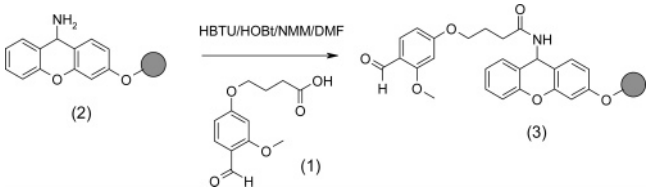


FIGURE 3. Coupling of MeOBAL to Sieber amide resin.

was then cleaved from the hyper acid labile linker with a low concentration of acid. Under these conditions (typically 1% TFA, 5% TES in DCM) the aldehyde linker is completely stable (data not published).

The construct of choice to define the limitations of the commercially available linker is shown below (Figure 4). Valine was chosen as a typical sterically hindered amine as subsequent acylation could be problematic with an electron deficient or structurally hindered acid. A number of polymer supported reductive alkylation protocols have been described in the literature.[7−9] Our method of choice utilized sodium cyanoborohydride in 1% AcOH/DMF for 18 h.

To ensure that quantitative reductive alkylation had occurred, a small portion of resin was cleaved and the resulting filtrate analyzed by HPLC and mass spectrometry techniques. Only one major peak was seen by HPLC, and electrospray mass spectrometry (ESI) confirmed this to be the predicted product (**5**). The HPLC chromatogram (Figure 5) showed no evidence of the starting aldehyde linker.

Following successful reductive alkylation, acylation was attempted with use of typical peptide synthesis techniques. To test the scope of this resin-linker construct, several acids were chosen for coupling with varying steric and electronic properties (Figure 6).

Each acid was mixed with **4** for 64 h, using HBTU as an in situ coupling agent in the presence of NMM and HOBt in DMF. The degree of acylation was determined by analysis of the cleaved products, using analytical HPLC/ESI.

Evaluation of the data showed that for all the acids chosen (with the exception of acetic acid) <5% acylation had occurred (Table 1, entries 1−8). The major product in each case (>95%) was the linker-Val-OBu^t starting material (Val-OMe also gave <5% acylation, Table 1, entries 9 and 10). This provided conclusive evidence that acylation of sterically hindered amines bound to MeOBAL proceeded at very low rates.

More forcing reaction conditions were employed in an attempt to improve acylation onto the hindered valine. The symmetrical anhydride of *Z*-leucine was prepared and compared to the coupling of acetic anhydride. As expected the use of acetic

(4) (a) del Fresno, M.; Alsina, J.; Royo, M.; Barany, G.; Albericio, F. *Tetrahedron Lett.* **1998**, *38*, 2639. (b) Farrant, E.; Rahman, S. S. *Tetrahedron Lett.* **2000**, *41*, 5383. (c) Tolborg, J. F.; Jenson, K. J. *Chem. Commun.* **2000**, 147. (d) Giovannoni. J.; Subra, G.; Amblard, M.; Martinez, J. *Tetrahedron Lett.* **2001**, *42*, 5389. (e) Herpin, T. F.; Van Kirk. K. G.; Salvino, M. J.; Yu, S. T.; Labaudiniere, R. F. *J. Comb. Chem.* **2000**, *2*, 513. (f) Estep, K. G.; Neipp, C. E.; Stephens Stramiello, L. M.; Adam, M. D.; Allen, M. P.; Robinson, S.; Roskamp, E. J. *J. Org. Chem.* **1998**, *63*, 5300.

(5) (a) Smith, J. M.; Krchnak, V. *Tetrahedron Lett.* **1999**, *40*, 7633. (b) Muzurov, A. *Bioorg. Med. Chem. Lett.* **2001**, *10*, 67.

(6) Fenwick, A. E.; Garnier, B.; Gribble, A. D.; Ife, R. J.; Rawlings, A. D.; Witherington, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 195.

(7) Ho, P. T.; Chang, D.; Zhong, J. W. X.; Musso, G. F. *Peptide Res.* **1993**, *6*, 10.

(8) Coy, D. H.; Hocart, S. J.; Sasaki, Y. *Tetrahedron* **1988**, *44*, 835.

(9) (a) Gordon, D. W.; Steele, J. *Biorg. Med. Chem. Lett.* **1995**, *5*, 47. (b) Szardenings, A. K.; Burkoth, S.; Look, G. C.; Campbell, A. *J. Org. Chem.* **1996**, *61*, 6720. (c) Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849.

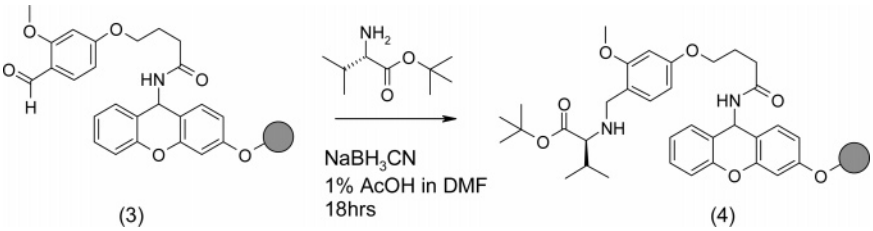


FIGURE 4. Valine-OBu^t reductively alkylated onto "double linker" to give construct (**4**).

TABLE 1. Acylation Reactions with MeOBAL (reaction time 64 h)

entry	amine reductively alkylated	coupling reagent	coupling additive	coupling base	coupling solvent	acid coupled	% starting amine remaining
1	valine-OBu^t	HBTU	HOBt	NMM	DMF	*Z*-Val-OH	>95
2	valine-OBu^t	HBTU	HOBt	NMM	DMF	*Z*-Leu-OH	>95
3	valine-OBu^t	HBTU	HOBt	NMM	DMF	*o*-anisic	>95
4	valine-OBu^t	HBTU	HOBt	NMM	DMF	*p*-anisic	>95
5	valine-OBu^t	HBTU	HOBt	NMM	DMF	*o*-nitrobenzoic	>95
6	valine-OBu^t	HBTU	HOBt	NMM	DMF	*p*-nitrobenzoic	>95
7	valine-OBu^t	HBTU	HOBt	NMM	DMF	acetic	<25
8	valine-OBu^t	HBTU	HOBt	NMM	DMF	diphenylacetic	>95
9	valine-OMe	HBTU	HOBt	NMM	DMF	*Z*-Val-OH	>95
10	valine-OMe	HBTU	HOBt	NMM	DMF	*Z*-Leu-OH	>95
11	valine-OBu^t	acetic anhydride		DIPEA	DCM	acetic anhydride	<5
12	valine-OBu^t	symmetrical anhydride of *Z*-leucine			DCM	*Z*-Leu-OH	>85

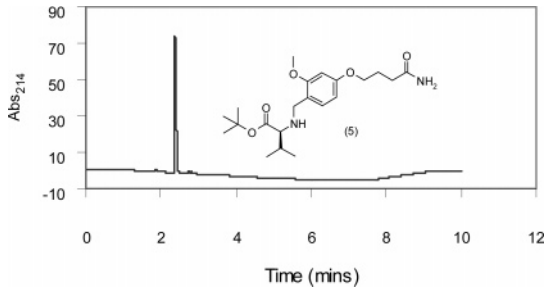


FIGURE 5. HPLC chromatogram of sample 5 from resin cleavage.

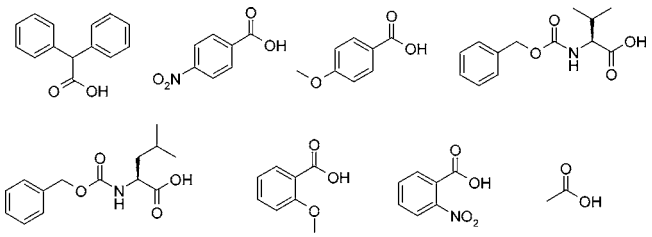
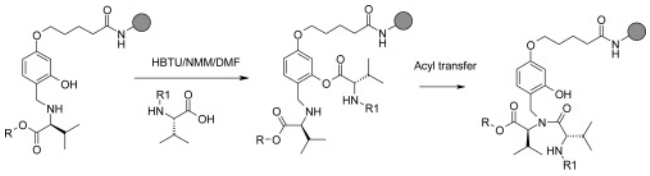


FIGURE 6. Selected acids for acylation onto H-Val-(OBu^t)-resin (**4**).

anhydride led to almost quantitative acylation (Table 1, entry 11). However, the use of the highly electrophilic symmetrical anhydride of the more hindered *Z*-leucine yielded <15% of the desired acylated product by HPLC area (Table 1, entry 12). HATU activation was also attempted as reported in the literature[3] but this gave little improvement over HBTU activation (results not shown). Almost identical results were observed when using BAL and Di-MeOBAL (Figure 1). Indole BAL (Figure 1) proved worse still, with only partial reductive alkylation being noted even after resubmission of the resin to the reductive conditions.

Having established the limitations of the commercially available linkers, work commenced on the investigation of a new

SCHEME 3. Oxygen−Nitrogen Acyl Transfer



aldehyde linker,[10] 4-(4-formyl-3-hydroxyphenoxy)pentanoic acid (OHBAL, Hydroxy, Backbone Amide Linker) (**6**) that would potentially overcome these problems (Scheme 4). We have described the synthesis of **6** in a patent,[10] but no experimental details of its use were included. Limited application of a similar linker has been reported.[11] In this work, simple unhindered amines were attached via reductive alkylation and coupled with simple amino acids. No demanding coupling reactions with respect to steric or electronic considerations were reported.

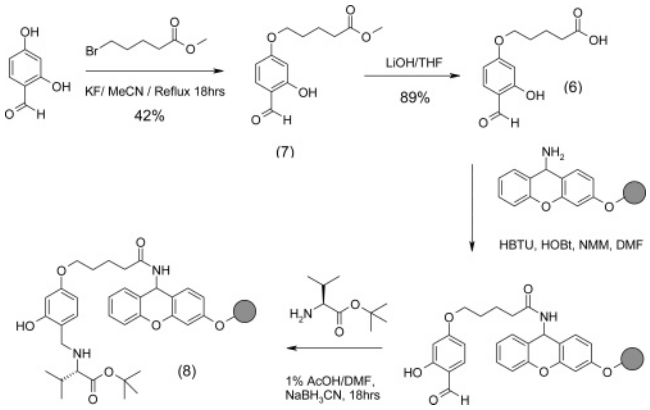
It was envisaged that linker **6** could be simply attached to any free amine resin via typical SPPS conditions (HBTU, HOBt, NMM, DMF, e.g., Figure 3).

When OHBAL is used, acylation with both electron poor and sterically hindered acids should be possible via initial acylation of the 3-hydroxy function followed by O to N acyl transfer of the incoming acid.[12] This concept is illustrated in Scheme 3.

The synthetic route to OHBAL itself is outlined in Scheme 4. Selective alklyaltion was achieved with conditions described by Mendelson et al.[13] Methyl 5-bromopentanoate, spray dried potassium fluoride, and 2,4-dihydroxybenzaldehyde were heated under reflux in acetonitrile. The crude methyl ester (**7**) was then purified by flash chromatography to give a white solid in good

(10) Johnson, T.; Quibell, M. *W.* Patent Appl. WO 98/17628 (File date: 1997).
(11) Okayama, T.; Burritt, A.; Hruby, V. J. *Org. Lett.* **2000**, *2*, 1787.
(12) Johnson, T.; Quibell, M.; Owen, D.; Sheppard, R. C. *J. Chem. Soc., Chem. Commun.* **1993**, 369.
(13) Mendelson, W. L.; Holmes, M.; Dougherty, J. *Synth. Commun.* **1996**, *26*, 593.

SCHEME 4. Synthesis of 5-(4-Formyl-3-hydroxyphenoxy)-pentanoic Acid (OHBAL) (6) and Subsequent Attachment to the Poylmer Support (8)



yield. Hydrolysis of the ester via treatment with lithium hydroxide in THF gave the desired acid (**6**) in good yield. Once this had been isolated, the construct **8** was prepared on the solid phase in a manner analogous to that used earlier to assemble construct **4** from MeOBAL (**1**) (Figure 4).

Following acidic cleavage of a small portion of resin, HPLC analysis revealed two peaks at 3.0 and 3.2 min in a relative ratio of 3:2. Electrospray MS confirmed the major component to be that of the desired product (**9**) and the minor product to be the imine (**10**) (Figure 7). This phenomenon was not noted when using the commercial linker and interestingly, Okayama et al.[11] did not report problems during reductive alkylation even though an identical 2-hydroxyl functionality was present on their linker. A variety of reported reductive alkylation conditions were employed, including those used by Okayama et al.,[11] and in each case incomplete alkylation was seen with the hindered amine. Even resubmission of the resin to the reductive conditions failed to isolate the desired amine quantitatively.

This discovery led to a modification to the design and synthesis of the new linker. It was proposed that temporary, orthogonal protection of the hydroxyl function would facilitate quantitative reductive alkylation. The revised structure and synthetic route are outlined in Scheme 5. Methyl 5-(4-formyl-3-hydroxyphenoxy)pentanoate (**7**), allyl bromide, and cesium carbonate were stirred at room temperature in dry acetonitrile for 3 h. The resulting crude product was purified by flash chromatography to yield a white solid ester (**11**) in high yield. Hydrolysis of the ester via treatment with lithium hydroxide in THF gave the desired acid (**12**) in excellent yield (ALOBAL, AllylOxy Backbone Amide Linker).

Pleasingly, reductive alkylation was found to occur quantitatively. A small portion of resin was cleaved and the filtrate analyzed by HPLC/ESI. One major peak at 3.1 min was identified as the desired product (**14**) (Supporting Information) and there was no evidence of the starting aldehyde linker.

The allyl protecting group was subsequently removed with a mixture of tetrakistriphenylphosphine palladium(0) and phenylsilane in DCM.[14] To ensure quantitative deprotection had occurred, a small portion of resin was cleaved and analyzed by HPLC/ESI. One major peak at 2.6 min was identified as the desired product (**15**) (Supporting Information) and there was no evidence of the starting material.

(14) Martinez, P. G.; Dessolin, G. F.; Albericio, F. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2871.

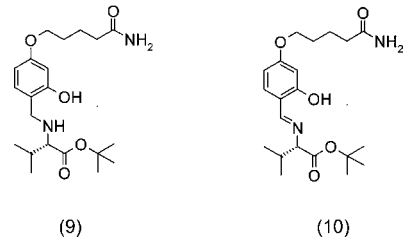


FIGURE 7. Two potential products derived from reductive alkylation with OHBAL.

With use of the same eight carboxylic acid probes as previously (Figure 6) and utilizing identical standard coupling chemistry, the synthetic utility of the resin was tested. The percentage acylation was determined by analysis of the cleaved products by HPLC/ESI.

The data clearly showed >80% acylation had occurred for all eight carboxylic acid probes (Table 2, entries 1−8) compared to <5% for the commercial linkers under identical conditions (Table 1). This provided conclusive evidence that the De-ALOBAL had overcome the synthetic limitations that were seen when using the commercial linkers for amide bond formation. An example of one of the HPLC chromatograms is shown in Figure 8.

The results clearly show the linker to be an extremely useful tool for the formation of amide bonds. Both electron poor and electron rich acids were coupled quantitatively to the sterically hindered amines. Of particular note is the successful acylation of a β-branched amino acid (*Z*-Val-OH) to the already sterically hindered Val-OBu^t and Val-OMe (Table 2, entries 1 and 10). One minor limitation of the linker was seen when coupling an ortho-substituted benzoic acid. Both *o*-anisic acid and 2-nitrobenzoic acid (Table 2, entries 3 and 5) showed slightly reduced acylation. A comparison of the degree of acylation with ortho/para anisic acids and ortho/para nitro benzoic acids (electron donating verses electron withdrawing) showed preference for the latter. Full ^{1}H and ^{13}C NMR characterization of one of the compounds prepared (**16**) (Table 2, entry 7) is detailed in the Experimental Section.

Having used the dual linker system to demonstrate the enhanced reactivity of the linker, the synthetic utility of this approach was investigated by using ALOBAL directly attached to the polymer (**17**) (Figure 9).

By using the same carboxylic acid probes as before (Figure 6) and utilizing identical standard coupling chemistry, seven amides were prepared. Each was isolated by treatment of the resin with 95% TFA, 5% TES. After workup the crude samples were lyophilized and accurate weights determined. The yields of the compounds (Table 3) were calculated by weight based on the initial loading of the commercially available resin. Purity was determined by HPLC peak area.

The purities of the amides were consistent with the data observed with the dual linker system, but the overall yields were a little lower than expected.

To test further the synthetic utility of the new linker, O to N acyl transfer was attempted with 4-methoxy aniline since as a class, anilines are poor nucleophiles compared to alkylamines.

The reductive alkylation chemistry described earlier for valine worked quantitatively with the ALOBAL and MeOBAL and worked well when using the Di-OMeBAL but did gave rise to minor impurities. Acylation with two acids (*Z*-valine-OH and *p*-nitrobenzoic acid), using HBTU chemistry, for both 24 and

SCHEME 5. Synthesis of 5-(3-Allyloxy-4-formylphenoxy)pentanoic Acid (ALOBAL) and Subsequent Attachment to the Solid Support (13)

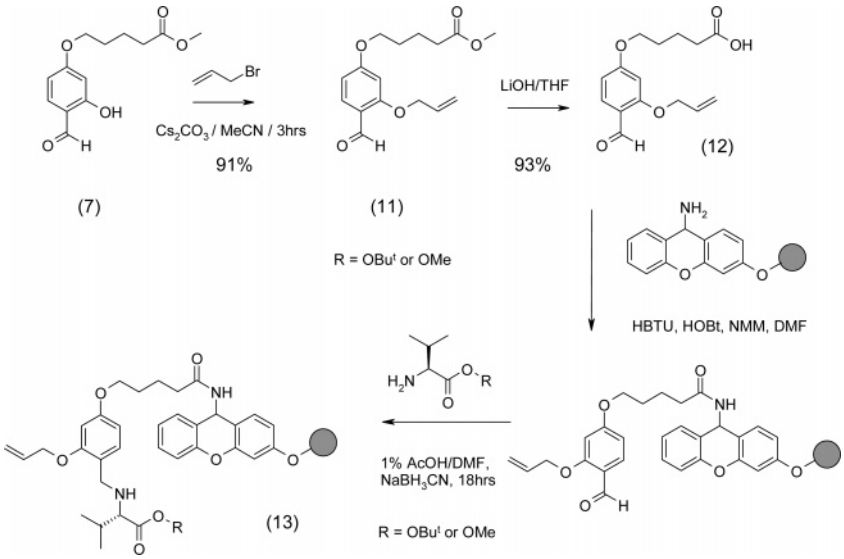


TABLE 2. Acylation Reactions with De-ALOBAL (reaction time 64 h)

entry	amine reductively alkylated	coupling reagent	coupling additive	coupling base	coupling solvent	acid coupled	% starting amine remaining
1	valine-OBu^t	HBTU	HOBt	NMM	DMF	*Z*-Val-OH	10
2	valine-OBu^t	HBTU	HOBt	NMM	DMF	*Z*-Leu-OH	7
3	valine-OMe	HBTU	HOBt	NMM	DMF	*o*-anisic	17
4	valine-OMe	HBTU	HOBt	NMM	DMF	*p*-anisic	2
5	valine-OMe	HBTU	HOBt	NMM	DMF	*o*-nitrobenzoic	10
6	valine-OMe	HBTU	HOBt	NMM	DMF	*p*-nitrobenzoic	1
7	valine-OBu^t	HBTU	HOBt	NMM	DMF	*p*-nitrobenzoic	2
8	valine-OMe	HBTU	HOBt	NMM	DMF	acetic	1
9	valine-OMe	HBTU	HOBt	NMM	DMF	diphenylacetic	1
10	valine-OMe	HBTU	HOBt	NMM	DMF	*Z*-Val-OH	9
11	valine-OMe	HBTU	HOBt	NMM	DMF	*Z*-Leu-OH	5

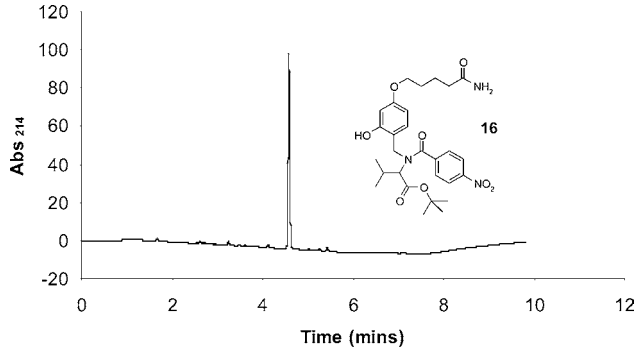


FIGURE 8. HPLC chromatogram of sample from resin cleavage (**16**) (entry 6, Table 2).

64 h then followed. As before, reductive alkylation with Indole BAL was not quantitative and so acylation experiments were not attempted. Following cleavage of the resins, the filtrates were analyzed by HPLC and electrospray MS. The results are tabulated below (Table 4).

The tabulated data clearly show a difference in performance of the three linkers. Interestingly, the rate of acylation with the Di-MeOBAL was superior to that of the MeOBAL linker (Table 4, entry 5 vs 3 and entry 6 vs 4), although neither commercial linker was as effective as ALOBAL.

One final test for the ALOBAL involved replacing 4-methoxyaniline (**25**) with the less nucleophilic 4-chloroaniline (**26**).

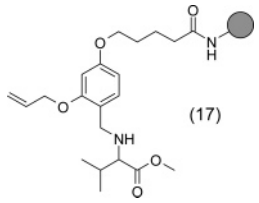


FIGURE 9. Valine-OMe reductively alkylated onto ALOBAL to give construct **17**.

The reductive alkylation chemistry described earlier for valine was found to be inappropriate for this electron poor aniline. Therefore, an alternative method was used involving dibutyltin dichloride as a catalyst and phenysilane as reductant.[15] Reductive alkylation was quantitative with all three linkers. Acylation was carried out for 64 h with 4-nitrobenzoic acid. The results are given in Table 5.

The data showed no acylated product when using the MeOBAL or Di-MeOBAL but quantitative acylation was seen with De-ALOBAL.

As before, the chemistry was then repeated with the ALOBAL directly attached to the polymer (Figure 10). Acylation was carried out for 64 h.

Following solid-phase synthesis, the amides were isolated by treatment of the resin with 95% TFA, 5% TES. After workup

(15) Apodaca, R.; Xiao, W. *Org. Lett.* **2001**, *3*, 1745.

TABLE 3. Yield and Purity of Amides Prepared with ALOBAL

compd	amine reductively alkylated	coupling reagent	coupling additive	coupling base	coupling solvent	acid coupled	% yield	% purity
18	valine-OMe	HBTU	HOBt	NMM	DMF	*Z*-Val-OH	66	84
19	valine-OMe	HBTU	HOBt	NMM	DMF	*Z*-Leu-OH	87	95
20	valine-OMe	HBTU	HOBt	NMM	DMF	*o*-anisic	68	93
21	valine-OMe	HBTU	HOBt	NMM	DMF	*p*-anisic	47	78
22	valine-OMe	HBTU	HOBt	NMM	DMF	*o*-nitrobenzoic	51	84
23	valine-OMe	HBTU	HOBt	NMM	DMF	*p*-nitrobenzoic	73	91
24	valine-OMe	HBTU	HOBt	NMM	DMF	diphenylacetic	72	89

TABLE 4. Acylation Reactions with Various Aldehyde Linkers (reaction time 24 & 64 h)

entry	linker	amine reductively alkylated	coupling reagent	coupling additive	coupling base	coupling solvent	acid coupled	% amine converted (24 and 64 h)
1	De-ALOBAL	*p*-anisidine	HBTU	HOBt	NMM	DMF	*Z*-Val-OH	100 and 100
2	De-ALOBAL	*p*-anisidine	HBTU	HOBt	NMM	DMF	*p*-nitrobenzoic	100 and 100
3	2-MeOBAL	*p*-anisidine	HBTU	HOBt	NMM	DMF	*Z*-Val-OH	11 and 28
4	2-MeOBAL	*p*-anisidine	HBTU	HOBt	NMM	DMF	*p*-nitrobenzoic	18 and 33
5	Di-MeOBAL	*p*-anisidine	HBTU	HOBt	NMM	DMF	*Z*-Val-OH	16 and 53
6	Di-MeOBAL	*p*-anisidine	HBTU	HOBt	NMM	DMF	*p*-nitrobenzoic	21 and 73

TABLE 5. Acylation Reactions (64 h) with Various Aldehyde Linkers

entry	linker	amine reductively alkylated	coupling reagent	coupling additive	coupling base	coupling solvent	acid coupled	% amine converted (64 h)
1	De-ALOBAL	*p*-chloroaniline	HBTU	HOBt	NMM	DMF	*p*-nitrobenzoic	98
2	2-MeOBAL	*p*-chloroaniline	HBTU	HOBt	NMM	DMF	*p*-nitrobenzoic	2
3	Di-MeOBAL	*p*-chloroaniline	HBTU	HOBt	NMM	DMF	*p*-nitrobenzoic	5

TABLE 6. Yield and Purity of Amides Prepared with ALOBAL

compd	amine reductively alkylated	coupling reagent	coupling additive	coupling base	coupling solvent	acid coupled	% yield	% purity
27	*p*-methoxyaniline	HBTU	HOBt	NMM	DMF	*p*-nitrobenzoic	85	94
28	*p*-methoxyaniline	HBTU	HOBt	NMM	DMF	*Z*-Val-OH	76	87
29	*p*-chloroaniline	HBTU	HOBt	NMM	DMF	*p*-nitrobenzoic	76	91

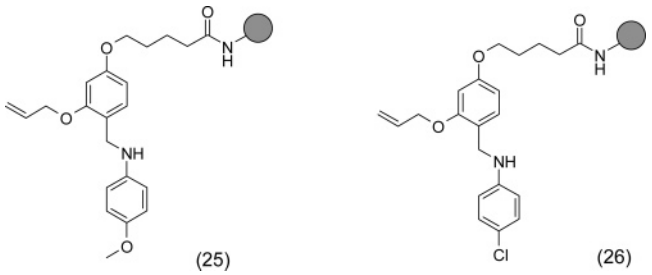


FIGURE 10. Anilines reductively alkylated onto ALOBAL to give resins (**25** and **26**).

the crude samples were lyophilized and accurate weights determined. The yield and purity (HPLC) of the compounds are shown in Table 6.

Summary

A novel linker (ALOBAL) for the solid phase synthesis of moieties linked through an amide bond has been prepared. This linker overcomes the limitations of similar commercially available handles in facilitating the quantitative acylation of both hindered (e.g., valine) and electron poor (e.g., anilines) secondary amines via *O*−*N* acyl transfer. The use of a double linker strategy enabled individual steps in the overall method to be monitored and optimized readily. Initial problems with the reductive alkylation step have been solved and the acylation reaction reduced to a routine coupling reaction with reagents/methods typically used in peptide chemistry for amide bond formation. Future work will be directed toward expanding the range of structural motifs that can be prepared by utilizing the unique O to N acyl transfer mechanism of De-ALOBAL.

Experimental Section

Acylation Experiments. All amide bonds were formed with the *standard acylation protocol*: HBTU (5 equiv), HOBt (5 equiv), and test acid (5 equiv) were pre-dissolved in a minimum volume of DMF and added to DMF swollen resin (1 equiv). *N*-Methylmorpholine (NMM) (10 equiv) was then added and the resin gently agitated for 64 h. When an alternative activating agent or base was used, exactly the same protocol was followed. Following acylation, resins were washed (20 mL/mmol resin) with standard washing protocol A: DMF (× 10), 5% hydrazine/DMF (10 min), DMF (× 10), MeOH, DCM (10 × each solvent) and dried for 2 h in vacuo.

Sieber Linker Cleavage. Cleavage was achieved with standard cleavage protocol A, using 10 mg of resin with gentle agitation: 1% TFA, 5% triethysilane (TES), and 94% DCM (total volume 2 mL) for 5 min. The filtrate was treated with an equal volume of brine and after separation, the organics were sparged with nitrogen to obtain a sample that was immediately analyzed by RP-HPLC and ES-MS.

Strong Acid Cleavage. Cleavage was achieved with standard cleavage protocol B, using 1.0 g of resin with gentle agitaion: 95% TFA, 5% TES (total volume 5 mL) for 90 min. The filtrate was concentrated in vacuo and taken up in acetonitrile/water (1:1) before lyophilization.

Symmetrical Anhydride Formation. Fmoc-Leu-OH (0.1 mmol, 35.3 mg) was dissolved in 2 mL of DCM. 1,3-Diisopropylcarbo-

diimide (0.05 mmol, 7.8 μL) was added and the solution was cooled to 0 °C for 45 min. The urea precipitate was filtered off and the filtrate added immediately to the resin.

Synthesis of Resin 3. 9-Fmoc-amino-xanthen-3-yloxy-Merrifield resin (Sieber, Novabiochem) was suspended in DMF (5 min) and then treated with a minimum volume of 20% piperidine in DMF (1 × 2 min, 1 × 5 min). After thorough washing (DMF × 10) linker (1) was coupled using the standard acylation protocol described above. The resin was then washed following standard washing protocol A and cleaved with standard cleavage protocol A.

Synthesis of Resin 4. Synthesis of resin **4** was carried out with *standard reductive alkylation protocol A*: The amine (either hydrochloride salt or free base) (5 equiv) was predissolved in the minimum volume of 1% acetic acid in DMF and added to resin **3**. Sodium cyanoborohydride (5 equiv) was then added and the resin was agitated for 18 h. After this time the resin was washed (20 mL/mmol resin) with standard washing protocol A and dried for 2 h in vacuo.

Synthesis of Compound 5. Resin **4** was cleaved with standard cleavage protocol A to generate compound **5** (Figure 5). HPLC: Peak 1 R_t 2.38 min (92%). *m*/*z* (ESMS) 395.12 (MH^+, 100%).

Preparation of 5-(4-Formyl-3-hydroxyphenoxy)pentanoic Acid (6). Compound **7** (1.0 g, 3.95 mmol) was dissolved in dioxan (10 mL) and to this was added 1 M lithium hydroxide (10 mL). The reaction was stirred at room temperature for 2 h. After cooling, the reaction mixture was concentrated in vacuo and then transferred to a separating funnel. EtOAc (50 mL) was added and the mixture was washed successively with H_2O (3 × 25 mL) and 1 M KOH (3 × 25 mL). The organic layer was dried over Na_2SO_4 and the solvent removed in vacuo to yield a white solid (0.837 g, 89%), mp 88−89 °C. HPLC: Peak 1 R_t 2.82 min (97%). Found: C, 60.53; H, 5.86. Calcd for $C_{12}H_{14}O_5$: C, 60.50; H, 5.92. ν_{max}(film)/cm^{-1} 2948 (sat. CH), 1691 (CO_2H), 1631 (CHO). *m*/*z* (ESMS) 237.20 (M − H 56%), 137.09 [M − $(CH_2)_4$−CO_2H), 100]. *m*/*z* (EI) found 261.0739. $C_{12}H_{14}O_5Na$ requires 261.0732. δ_H (400 MHz; $CDCl_3$) 9.78 (1H, s), 9.70 (1H, s), 7.51 (1H, d, J = 8.5 Hz), 6.53 (1H, dd, J = 8.5, 2.3 Hz), 6.39 (1H, d, J = 2.3 Hz), 4.00 (2H, t, J = 5.8 Hz), 2.40−2.37 (2H, m), 1.85−1.71 (4H, m). δ_C (400 MHz; $CDCl_3$) 194.5, 176.2, 166.6, 164.2, 135.1, 115.6, 108.2, 100.9, 68.1, 33.3, 28.3, 21.5.

Preparation of Methyl 5-(4-Formyl-3-hydroxyphenoxy)pentanoate (7). 2,4-Dihydroxybenzaldehyde (10 g, 7.5 mmol), methyl 5-bromopentanoate (12.9 mL, 1.2 equiv, 9 mmol), and spray dried potassium fluoride (8.85 g, 1.0 equiv, 7.5 mmol) were dissolved in dry acetonitrile (100 mL) and heated under reflux for 18 h (moisture excluded with calcium chloride drying tube). After cooling, the reaction mixture was concentrated in vacuo and then transferred to a separating funnel. EtOAc (150 mL) was added and washed successively with H_2O (3 × 75 mL) and 1 M KOH (3 × 75 mL). The organic layer was dried over Na_2SO_4 and the solvent removed in vacuo to yield a brown solid (16.3 g, 75%). TLC: 30%EtOAc/70%heptane: 3 spots, minor 1 R_f 0.32 (starting material), minor 2 R_f 0.1 (acid, hydrolyzed from methyl ester), major R_f 0.36 desired product. HPLC: Peak 1 R_t 2.81 min (17%), peak 2 R_t 2.81 min (30%), peak 3 R_t 3.78 min (51%). Purification by flash chromatography. The product was dry loaded and eluted with 20% EtOAc/heptane. Appropriate fractions were combined and concentrated in vacuo to afford the three compounds stated above, all as white/cream solids. Desired product (7.6 g, 42%), mp 65−66 °C. Found: C, 61.90; H, 6.37. Calcd for $C_{13}H_{16}O_5$: C, 61.90; H, 6.39. ν_{max}(film)/cm^{-1} 2949, 1734, 1630. *m*/*z* (ESMS) 251.23 (M − H 100%), 137.10 [M − $(CH_2)_4$−CO_2H), 11.83]. *m*/*z* (EI) found 275.0895. $C_{13}H_{16}O_5Na$ requires 275.0890. δ_H (400 MHz; $CDCl_3$) 11.45 (1H, s), 9.70 (1H, s), 7.40 (1H, d, J = 8.50 Hz), 6.50 (1H, dd, J = 8.5 Hz, J = 2.3 Hz), 6.40 (1H, d, J = 2.3 Hz), 4.00 (2H, t, J = 5.8 Hz), 3.70 (3H, s), 2.42−2.38 (2H, m), 1.88−1.76 (4H, m). δ_C (400 MHz; $CDCl_3$) 188.5, 173.9, 165.6, 162.9, 130.7, 119.4, 106.7, 99.6, 68.0, 51.8, 33.8, 28.9, 21.7.

Preparation of Methyl 5-(3-Allyloxy-4-formylphenoxy)pentanoate (11). Compound **7** (0.5 g, 1.98 mmol) was dissolved in dry acetonitrile (10 mL). To this was added allyl bromide (1.1 equiv, 2.18 mmol, d = 1.4, 0.188 mL) and cesium carbonate (2.0 equiv, 3.96 mmol, 1.29 g). The reaction was stirred at room temperature for 2 h. TLC: 20% EtOAc/heptane 1 spot. R_f 0.19 (desired product). HPLC peak 1 R_t 4.13 min (97%). The reaction mixture was filtered through Celite, concentrated in vacuo, and then transferred to a separating funnel. EtOAc (50 mL) was added and washed successively with 1 M $KHSO_4$ (3 × 20 mL) and brine (1 × 30 mL). The organic layer was dried over $MgSO_4$ and the solvent removed in vacuo to yield 0.527 g of a cream colored solid (91%), mp 39−40 °C. Found: C, 65.54; H, 6.85. Calcd for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90. ν_{max}(film)/cm^{-1} 2949, 1735, 1677, 1601, 1260; *m*/*z* (ESMS) 291.23 (M − H 100%), 251.20 (M − CH_2−CH═CH_2), 177.15 [M − $(CH_2)_4$−CO_2H), 10]. *m*/*z* (EI) found 315.1208. $C_{16}H_{20}O_5Na$ requires 315.1202. δ_H (400 MHz; $CDCl_3$) 10.30 (1H, s) 7.88 (1H, d, J = 8.5 Hz), 6.54−6.50 (1H, m), 6.45 (1H, d, J = 2.3 Hz), 6.13−6.01 (1H, m), 5.45 (1H, dq, J = 17.0 Hz, J = 1.50 Hz), 5.33 (1H, dq, J = 10.5 Hz, J = 1.5 Hz), 4.62 (2H, dt, J = 5.00 Hz, J = 1.5 Hz), 4.02 (2H, t, J = 5.85 Hz), 3.70 (3H, s), 2.42−2.39 (2H, m), 1.85−1.71 (4H, m). δ_C (400 MHz; $CDCl_3$) 188.5, 173.9, 165.6, 162.9, 132.5, 130.7, 119.4, 118.3, 106.7, 99.6, 69.4, 68.0, 51.8, 33.8, 28.7, 21.7.

Preparation of 5-(3-Allyloxy-4-formylphenoxy)pentanoic Acid (12). The crude solid (**11**) (0.527 g, 2.08 mmol) was dissolved in dioxan (10 mL) and to this was added 1 M lithium hydroxide (5 mL). The reaction was stirred at room temperature for 2 h. After cooling, the reaction mixture was concentrated in vacuo and then transferred to a separating funnel. EtOAc (50 mL) was added and washed successively with H_2O (3 × 25 mL) and 1 M KOH (3 × 25 mL). The organic layer was dried over Na_2SO_4 and the solvent removed in vacuo to yield a white solid (0.466 g, 93%), mp 110−111 °C. HPLC: Peak 1 R_t 2.93 min (96%). Found: C, 65.63; H, 6.45. Calcd for $C_{15}H_{18}O_5$: C, 65.74; H, 6.52. ν_{max}(film)/cm^{-1} 2952, 1706, 1676, 1604, 1266. *m*/*z* (ESMS) 277.25 (M − H 100%), 177.15 [M − $(CH_2)_4$−CO_2H), 98.6]. *m*/*z* (EI) found 301.1052. $C_{15}H_{18}O_5Na$ requires 301.1047. δ_H (400 MHz; $CDCl_3$) 10.30 (1H, s), 7.73 (1H, d, J = 9.3 Hz), 6.62−6.58 (1H, m), 6.52−6.48 (1H, m), 6.04−6.16 (1H, m), 5.45 (1H, dq, J = 17.00 Hz, J = 1.5 Hz), 5.30 (1H, dq, J = 10.54 Hz, J = 1.50 Hz), 4.55 (2H, dt, J = 5.0 Hz, J = 1.50 Hz), 4.02 (2H, t, J = 5.85 Hz), 2.41−2.38 (2H, m), 1.88−1.76 (4H, m). δ_C (400 MHz; $CDCl_3$) 188.7, 176.1, 166.4, 163.4, 132.9, 130.1, 118.8, 117.0, 107.2, 99.2, 69.2, 68.1, 33.4, 28.4, 21.5.

Synthesis of Compound 15. Resin **13** (1.0 equiv) was treated with 0.5 equiv of $(PPh_3)_4Pd(0)$ and 25 equiv of phenylsilane in DCM for 5 min. The resin was thoroughly washed following washing protocol B: DMF (10 × 5 mL), 0.5% DIPEA, 0.5% sodium diethyldithiocarbamate in DMF (15 min agitation), DMF (10 × 5 mL), DCM (10 × 5 mL). The procedure was then repeated and finally the resin dried for 2 h in vacuo. The resin was cleaved by using the standard cleavage protocol A to generate compound **15**. HPLC: Peak 1 R_t 2.58 min (91%). *m*/*z* (ESMS) 395.22 (MH^+, 100%).

Synthesis of Compound 16. Allyl protection was removed from resin **13** as described above. The resin was then reacted with 4-nitrobenzoic acid, using the standard acylation protocol. The resin was washed using standard washing protocol A and then cleaved using standard cleavage protocol A. The crude material was lyophilised from acetonitrile/water (1:1 v/v) to yield a yellow solid (Figure 8). HPLC: Peak 1 R_t 4.60 min (96%). *m*/*z* (ESMS) 544.37 (M + H 44.17%), 488.30 [M − Bu^t), 100%]. δ_H (400 MHz; $CDCl_3$) 8.29 (1H, d, J = 8.6 Hz), 7.67 (1H, d, J = 8.6 Hz), 7.15 (1H, d, J = 8.6 Hz), 6.46 (1H, s), 6.37 (1H, d, J = 8.6 Hz), 6.04 (1H, s), 5.64 (1H, s), 4.65 (1H, d, J = 14.8 Hz), 4.54 (1H, d, J = 14.8 Hz), 3.90−4.01 (2H, m), 3.71 (1H, d, J = 13.3 Hz), 2.35−2.45 (1H, m), 2.25−2.35 (2H, m), 1.82−1.88 (2H, m), 1.49 (9H, s), 0.79 (1H, d, J = 6.2 Hz), 0.65 (1H, d, J = 6.2 Hz). δ_C (400 MHz;

$CDCl_3$) 176.5, 173.5, 168.5, 161.1, 157.9, 148.9, 141.4, 133.6, 128.8, 124.1, 113.9, 106.9, 103.1, 83.6, 69.8, 67.6, 43.9, 35.6, 28.71, 28.4, 28.2, 22.4, 20.0, 18.9.

Synthesis of Resin 17. NovaSyn (Novabiochem) TGR (1.0 g, 0.2 mmol) was suspended in DMF (5 min) and then treated with a minimum volume of 20% piperidine in DMF (1 × 2 min, 1 × 5 min). After thorough washing (DMF ×10) linker **12** was coupled using the standard acylation protocol. The resin was then washed using standard washing protocol A. After pre-swelling in DMF, HCl.Val-OMe was reacted using standard reductive alkylation protocol A and then washed using standard washing protocol A.

Synthesis of Compound 18.[16] Resin **17** was treated with 0.5 equiv of $(PPh_3)_4Pd(0)$ and 25 equiv of phenylsilane in DCM for 5 min. The resin was thoroughly washed using standard washing protocol B and the procedure repeated. *Z*-Valine was coupled using the standard acylation protocol. The resin was once again washed using standard washing protocol A and then subjected to the standard cleavage protocol B. The crude material was lyophilised from acetonitrile/water to yield a clear oil (0.048 g, 66%). HPLC: Peak 1 R_t 4.10 min (99%). *m*/*z* (ESMS) 365.03 (M + H 37.27%). *m*/*z* (EI) found 387.1904. $C_{19}H_{28}N_2O_5Na$ requires 387.1896. δ_H (400 MHz; $CDCl_3$) 7.38−7.22 (5H, m), 5.32 (1H, s) 5.16 (2H, m), 4.56 (1H, m), 4.31 (1H, m), 3.73 (3H, s), 2.08 (2H, m), 1.00−0.86 (12H, m). δ_C (400 MHz; $CDCl_3$) 173.0, 172.6, 156.8, 129.8−129.2, 67.4, 59.1, 57.8, 52.6, 30.8, 19.7−18.2.

Synthesis of Resin 26. NovaSyn (Novabiochem) TGR (1.0 g, 0.2 mmol) was suspended in DMF (5 min) and then treated with a minimum volume of 20% piperidine in DMF (1 × 2 min, 1 × 5 min). After thorough washing (DMF ×10) linker **12** was coupled using the standard acylation protocol. The resin was then washed using standard washing protocol A. After preswelling in DMF, 4-chloroaniline was reacted using reductive alkylation protocol B: The aniline (5 equiv) and dibutyltin dichloride (0.1 equiv) were dissolved in the minimum volume of dry THF and added to the resin. After gentle agitation (5 min) phenylsilane (5 equiv) was added and the resin agitated for 18 h. After this time the resin was washed (20 mL/mmol resin) using standard washing protocol A.

Synthesis of Compound 29.[22] Resin **26** was treated with 0.5 equiv of $(PPh_3)_4Pd(0)$ and 25 equiv of phenylsilane in DCM for 5 min. The resin was thoroughly washed using standard washing protocol B and the procedure repeated. 4-Nitrobenzioc acid was coupled using the standard acylation protocol. The resin was once again washed using standard washing protocol A and after drying in vacuo was subjected to the standard cleavage protocol B. The crude material was lyophilized from acetonitrile/water to yield a yellow solid (0.042 g, 76%). HPLC: Peak 1 R_t 5.22 min (99%). *m*/*z* (ESMS) 277.45 (M + H 43.37%). *m*/*z* (EI) found 299.6593. $C_{19}H_{28}N_2O_5Na$ requires 299.6652. δ_H (400 MHz; DMSO) 8.35 (2H, d, J = 8.78 Hz), 8.16 (2H, d, J = 9.28 Hz), 7.80 (2H, d, J = 8.78 Hz), 4.42 (2H, d, J = 9.28 Hz). δ_C (400 MHz; $CDCl_3$) 164.6, 149.9, 141.0, 138.4, 129.9, 129.32, 128.5, 124.2, 122.7.

Supporting Information Available: Full characterization of all numbered products along with detailed procedures for the additional reactions.[17−21] This material is available free of charge via the Internet at http://pubs.acs.org.

JO051742J

(16) Breit, B.; Laungani, A. C. *Tetrahedron: Asymmetry* **2003**, *14*, 3823−3826.

(17) Govindachari, T. R.; et al. *Tetrahedron* **1967**, *23*, 4811−4815.

(18) Suga, H.; Shi, X.; Ibata, T.; Kakehi, A. *Heterocycles* **2001**, *9*, 1711−1726.

(19) Fache, F.; Valot, F.; Milenkovic, A.; Lemaire, M. *Tetrahedron* **1996**, *52*, 9777−9784.

(20) Mitas, P.; Sedlak, M.; Kavalek, J. *J. Chem. Soc., Commun.* **1998**, *63*, 85−93.

(21) Mathis, C. A.; Wang, Y.; Holt, D. P.; Huang, G.-F.; Debnath, M. L.; Klunk, W. E. *J. Med. Chem.* **2003**, *46*, 2740−2754.

(22) Walczyna, R.; Sokolowski, J. *Pol. J. Chem.* **1984**, *58*, 791−804