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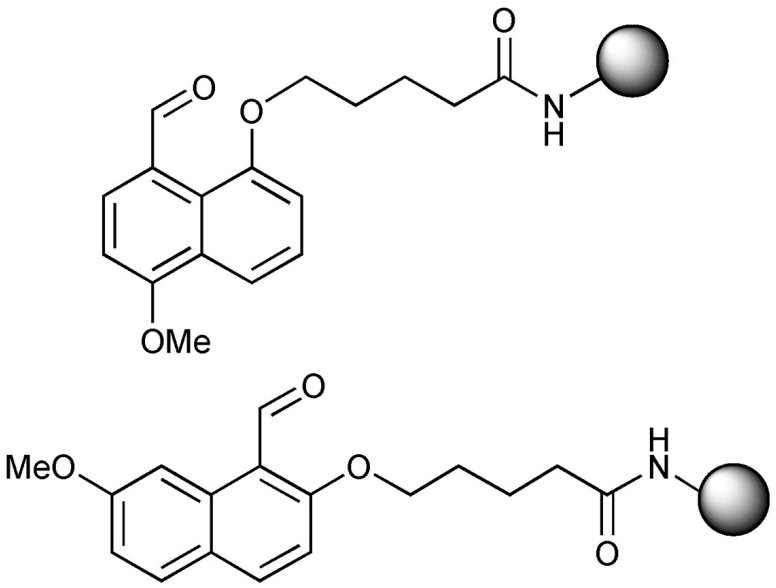

Two Dialkoxynaphthalene Aldehydes as Backbone Amide Linkers for Solid-Phase Synthesis

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Two new solid-phase handles for backbone amide anchoring based on regioisomeric dialkoxynaphthalene aldehydes (NALdehydes) were synthesized in five convenient steps from the corresponding commercially available dihydroxynaphthalenes. The two NALdehydes were coupled to an aminomethyl polystyrene support, the first monomer attached by efficient reductive amination, and the secondary amine acylated to form naphthalene amide linker (NAL-1 and NAL-2) anchoring. After on-resin synthesis, release of peptides was effected with TFA/H_2O (95:5), TFA/DCM (50:50), or low TFA concentrations. The properties of the NAL handles were evaluated in the solid-phase synthesis of a series of peptides, in which NAL-2 showed the best cleavage properties.

Introduction

The advent of combinatorial chemistry has been accompanied by an immense interest in solid-phase organic synthesis. A crucial part of solid-phase synthesis is the attachment to the polymeric support.[1,2] The first building block has to be attached efficiently, the linkage has to be stable to the subsequent chemical transformations, and conditions for release from the support should be compatible with the final products. Linkers (handles), which upon treatment with concentrated trifluoroacetic acid (TFA) release amides by C−N bond cleavage, have in most cases been designed from substituted benzyl, benzhydryl, and trityl derivatives as "core structures", with addition of substituents to fine-tune the acid-lability.[2,3] Development of new handles for solid-phase synthesis continues to be of great interest, and new core structures, in addition to the above-mentioned, could serve as starting points for the design of whole new families of handles. In Merrifield's original design for solid-phase peptide synthesis, the growing peptide chain was anchored through the C-terminal carboxyl while elongated from the N-terminal. However, many biological active peptides are either C-terminal modified or cyclic. The backbone amide linker (BAL) strategy was developed for the synthesis of C-terminal modified peptides.[4] In this strategy, the first amino acid is introduced by reductive amination, followed by acylation of the newly formed secondary amine. Thus, the growing peptide chain is anchored not through the C-terminal carboxyl, but through a backbone amide nitrogen, giving access to, in principle, any C-terminal modification. In the first implementation of this general strategy, amino acid derivatives were attached by convenient and reliable reductive amination to support-bound 5-(4-formyl-3,5-dimethoxyphenoxy)valeric acid,[4,5] forming a tris(alkoxy)benzylamine linkage. The BAL handle strategy has since also been applied to the synthesis of nitrogen-containing small organic molecules[4b,6] and oligosaccharides.[7] Less electron-rich analogues, such as dialkoxy[8] and monoalkoxy benzaldehydes,[8b] as well as an indole based handle,[9] have also been used as core structures in linkers for solid-phase synthesis by the BAL strategy. Together, BAL-type handles have found widespread use in combinatorial chemistry.

In this paper, we introduce and evaluate a novel class of backbone amide linkers based on dialkoxynaphthalene aldehydes.[10] Upon acidolysis, the carbenium ion is stabilized by delocalization through an aromatic system larger than a substituted benzyl derivative (more canonical structures can be shown for naphthalene than benzene).[11] Methoxy substituents on the aromatic core stabilize the carbenium ion further by resonance stabilization. Additionally, naphthalenes exhibit a steric strain associated with 1,8-disubstitution (peri effect), because 1,8-substituents are in closer proximity than the corresponding ortho substituents. We envisioned that acidolytic release of a compound from NAL-1 (naphthalene amide linker) could relieve some of the steric strain and, thus, promote the release.[12] In addition, these planar structures are easy to handle in synthesis. In the design of both NAL-1 and NAL-2, one of the methoxy substituents is located in close vicinity to the aldehyde (peri for NAL-1 and ortho for NAL-2) which allows regioselective demethylation under chelation control[5c] to liberate a hydroxy group for subsequent introduction of the spacer. The naphthalene handles can be synthesized conveniently from the corresponding dihydroxynaphthalenes, which are inexpensive, commercially available starting materials.

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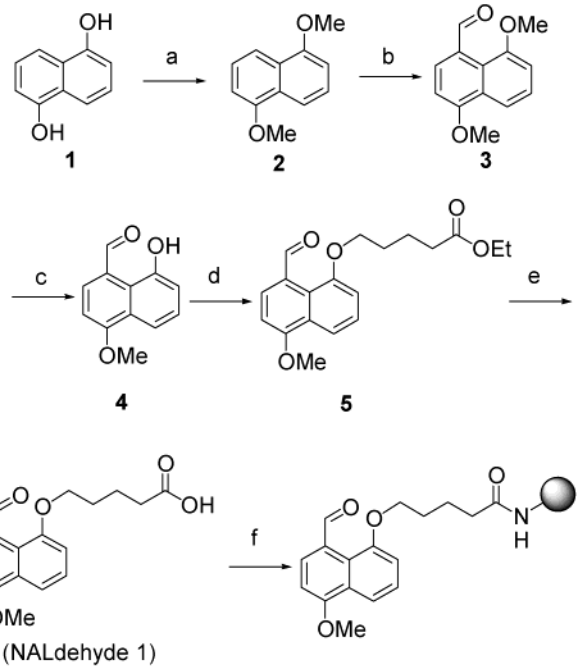
Scheme 1. Synthesis of NALdehyde-1[a]



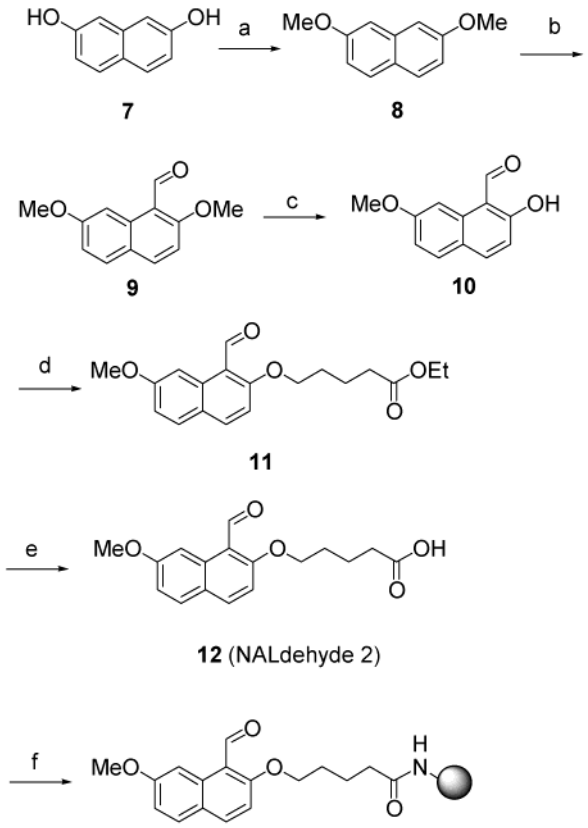
[a] Reaction conditions: (a) $(MeO)_2SO_2$, aq NaOH; (b) $POCl_3$, DMF, toluene, reflux, 5 h; (c) BBr_3, DCM, −60 °C, 1 h; (d) ethyl 5-bromovalerate, KO*t*Bu, DMF, 60 °C, 12 h; (e) aq NaOH, THF, rt, 12 h; (f) aminomethylated polystyrene, PyBOP, DIEA, DMF, rt, 16 h.

Results and Discussion

NAL-1 was synthesized from commercially available 1,5-dihydroxynaphthalene (**1**, Scheme 1) which was subjected to dimethylation, Vilsmeier−Haack formylation, and regioselective monodemethylation by chelation control according to published procedures.[13,14] Subsequently, naphthol derivative **4** (Scheme 1) was alkylated smoothly in 80% yield with ethyl 5-bromovalerate in DMF at 60 °C in the presence of potassium *tert*-butoxide as base. Product **5** was easy to isolate due to precipitation during aqueous workup. The ethyl ester was cleaved under mild conditions by aqueous sodium hydroxide in THF, giving 5-(8-formyl-5-methoxynaphthalene-1-yl-oxy)-pentanoic acid (**6**, NALdehyde-1) (in naming it "NALdehyde," we follow the precedent set by the name "PALdehyde"; see ref 4) in quantitative yield (Scheme 1).

Synthesis of NALdehyde-2 started out from commercially available 2,7-dihydroxynaphthalene (**7**), which was dimethylated to **8** in 66% yield, followed by Vilsmeier−Haack formylation in 78% by improving on literature procedures (Scheme 2).[15,16] Regioselective monodemethylation was performed in 80% yield, utilizing $AlCl_3$ as demethylation reagent.[17] Subsequently, the naphthol derivative **10** (Scheme 2) was alkylated with ethyl 5-bromovalerate analogous to NALdehyde-1 to furnish **11** in 73% yield. The ethyl ester was hydrolyzed in a manner similar to that for NALdehyde-1, giving 5-(1-formyl-7-methoxynaphthalene-2-yl-oxy)-pentanoic acid **12** (NALdehyde-2) in 76% yield. Anchoring of the NALdehydes to aminomethylated polystyrene (PS) solid support was achieved with benzotriazole-1-yl-oxy-tris-(pyrrolidino)-phosphonium hexafluoro phosphate (PyBOP) or *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HBTU) in the presence of diisopropylethylamine (DIEA).

Scheme 2. Synthesis of NALdehyde-2[a]



[a] Reaction conditions: (a) MeI, K_2CO_3, DMF, 50 °C, 16 h; (b) $POCl_3$, DMF, 60 °C, 16 h; (c) $AlCl_3$, DCM, rt, 16 h; (d) ethyl 5-bromovalerate, KO*t*Bu, DMF, 60 °C, 12 h; (e) aq NaOH, THF, rt, 12 h.; (f) aminomethylated polystyrene, PyBOP, DIEA, DMF, rt, 16 h.

Evaluation of NAL-1 and NAL-2 (Tables 1−3). In a first test of the efficiency of the new handles, we performed a short reaction sequence: (1) Anchoring of H-Ala-OMe was achieved by reductive amination to NALdehyde-1 PS to form a NAL-1 linkage. (2) Acylation of the secondary amine with N^{α}-Fmoc-Gly-OH proceeded under a variety of conditions. These acylation and reductive amination conditions for NAL-1 were then applied to NAL-2. (3) Relative acid-lability of the handles was evaluated by acidolytic release of the formed dipeptide, N^{α}-Fmoc-Gly-Ala-OMe from the resin under various conditions. HPLC−UV standard curves of the Fmoc chromophore were used to measure the amount of Fmoc-peptide released from the resin. Large differences in the overall yield of the protected dipeptide were observed, depending on the conditions for the individual steps in the solid-phase synthesis. This provided the following results (Tables 1−3).

Evaluation and Optimization of Solid-Phase Reductive Amination of NALdehyde PS with Ala-OMe·HCl (Table 1). The amino acid was anchored by reductive amination to the solid phase using various procedures at room temperature.[4] A 10-fold excess of the amine did not give any significant improvement, compared to a 2-fold excess, in the overall yield of the Fmoc-protected dipeptide in this system. However, in synthesis of other peptide sequences, a 10-fold excess of amine resulted in great improvement of the overall yield of peptide. In general, longer reaction times also gave better overall yields.

Table 1. Reductive Aminations of NALdehyde-1 Resin with H-Ala-OMe HCl Salt as a Function of Amount of Amine, $NaBH_3CN$, and Time[a,b]

entry	amine (equiv)	$NaBH_3CN$ (equiv)	time (h)	yield (%)
1	10	10	1	28
2	2	10	1	16
3	10	10	24	68
4	2	10	24	66

[a] Acylation with the symmetrical anhydride of N^{α}-Fmoc-Gly-OH: N^{α}-Fmoc-Gly-OH (10 equiv), DIPCDI (5 equiv) in DCM/DMF (9:1), 1 h. Cleavage of Fmoc-Gly-Ala-OMe from the resin: TFA/water (19:1, 1 mL pr.10 mg resin), 2 h. Monitoring and quantification by HPLC−UV at 265 nm. [b] See ref 19.

Table 2. Acylations of H−Ala-OMe Derivatized NAL-1 Resin as a Function of Coupling Reagent[a,b,c]

entry	coupling reagent	base	solvent	time (h)	yield (%)
1	DIPCDI		DMF	1	34
2[b]	HBTU	DIEA	DMF	1	25
3[b]	PyBOP	DIEA	DMF	1	10
4[b]	TFFH	DIEA	DMF	1	5
5	DIPCDI		DCM/DMF, 9:1	1	68
6[b]	HBTU	DIEA	DCM/DMF, 9:1	1	31
7[b]	PyBOP	DIEA	DCM/DMF, 9:1	1	7
8[b]	TFFH	DIEA	DCM/DMF, 9:1	1	24

[a] Reductive aminations were performed as follows: H-Ala-OMe (2 equiv) and $NaBH_3CN$ (10 equiv) for 24 h. Acidolytic cleavage of Fmoc-Gly-Ala-OMe from resin: TFA/H_2O (95:5, 1 mL pr. 10 mg resin), for 2 h. Monitoring and quantification by HPLC−UV at 265 nm. [b] N^{α}-Fmoc-Gly-OH (0.149 g, 0.50 mmol) and HBTU (0.190 g, 0.50 mmol), PyBOP (0.260 g, 0.50 mmol), or TFFH (0.132 g, 0.50 mmol) was placed in a filter syringe containing the H-Ala-OMe-derivatized NALdehyde resin (0.050 g, 0.050 mmol). DMF (2 mL) or DCM/DMF 9:1 (2 mL) was added, followed by DIEA (0.173 mL, 1.00 mmol). The reaction mixture was shaken for 1 h. The resin was washed with DMF (10×), DCM (5×), and MeOH and air-dried. [c] See ref 19.

Table 3. Acidolytic Cleavages of Fmoc-Gly-Ala-OMe from NAL-Derivatized Resin as a Function of Amount TFA and Time[a,b]

			yield (%)	
entry	TFA/DCM/H_2O	time (h)	NAL-1	NAL-2
1	95:0:5	1	81	61
2	95:0:5	2	84	65
3	95:0:5	24	48	79
4	50:50:0	1	82	69
5	5:95:0	1	30	26
6	5:95:0	24	21	70

[a] Reductive aminations were performed with H-Ala-OMe (10 equiv) and $NaBH_3CN$ (10 equiv) for 24 h. Acylations with the symmetrical anhydride were carried out with N^{α}-Fmoc-Gly-OH (10 equiv) and DIPCDI (5 equiv) in DCM/DMF (9:1) for 1 or 16 h. Monitoring and quantification by HPLC−UV (265 nm). [b] See ref 19.

Evaluation of Conditions for Acylation of H-(NAL-1-PS)Ala-OMe with Fmoc-Gly-OH (Table 2). Acylations of the secondary amine were carried out using two strategies, either with symmetrical anhydrides in DCM/DMF (9:1) or with coupling reagents, such as PyBOP, HBTU, and 1,1,3,3-tetramethyl-2-fluoroformamidinium hexafluorophosphate (TFFH)[18] in DMF or DCM/DMF (9:1). PyBOP, HBTU, and TFFH in DMF gave only modest overall yields of dipeptide.

Table 4. Acidolytic Cleavage of H-Tyr-Gly-Gly-Phe-Leu-OH from NAL-2 Derivatized Resin as a Function of TFA Content[a]

TFA/DCM/H_2O/TIS	time (h)	yield (%)
95:0:5:0	2	75
90:0:5:5	2	69
50:50:0:0	2	75
50:45:0:5	2	68
5:95:0:0	2	67
5:90:0:5	2	63

[a] Cleavage yields were determined by amino acid analysis (AAA) using Ala as internal reference amino acid (IRAA).

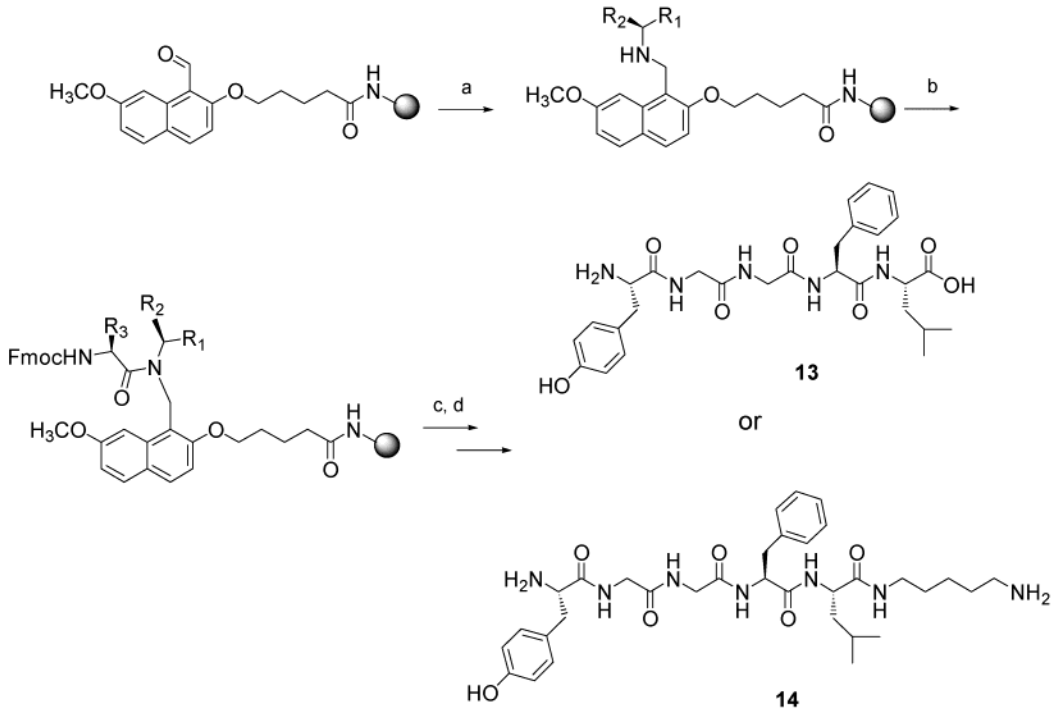
The very poor perfomance of PyBOP in DCM/DMF was attributed to low solubility of the coupling reagent in this solvent mixture (Table 2, entry 7). Good acylation yields of the secondary amine were obtained with DIPCDI in DCM/DMF (9:1). The acylation proceeded via the symmetrical anhydride and, as observed for BAL, the yield was greatly improved with decreasing polarity of the solvent.[4,5]

Evaluation of Conditions for Acidolytic Cleavage of Fmoc-Gly-Ala-OMe from NAL-PS Resins (Table 3). Cleavages were performed using either TFA/H_2O (95:5) or TFA/DCM mixtures, with TFA/H_2O giving slightly better overall yields. However, prolonged exposure to TFA/H_2O decreased the overall yield. Changing the TFA/DCM ratio from 50:50 to 5:95 also resulted in a lower yield (Table 3, entry 5).

The initial cleavage studies led to the conclusion that the NAL-1 handle gave slightly higher cleavage yields under "high-acid" conditions than NAL-2; hovewer, the NAL-2 handle had a higher acid lability than NAL-1 under "low-acid" conditions, TFA/DCM 5:95 (Table 3, entry 6). To elucidate the optimal cleavage conditions for NAL-2 further, the pentapeptide Leu-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH) was synthesized on solid phase, and the peptide was released from the resin under various conditions. The cleavage yields were determined by amino acid analysis (AAA), incorporating Ala as an internal reference amino acid (IRAA) between the resin and the handle. The cleavage yields were determined using the Leu/Ala ratio and corrected with the Leu/Ala ratio before cleavage (Table 4). Generally, there was a good correlation with the cleavage yields obtained for NAL-2 in the dipeptide model system analyzed by HPLC−UV. The amino acid analysis similarly led to the conclusion that the peptide could be released smoothly from the resin with either a TFA/H_2O 95:5 mixture or a TFA/DCM 50:50 mixture.

Finally, Leu-enkephalin was synthesized on solid-phase anchored NALdehyde-1 and -2 and was isolated in 35 and 56% crude yield, respectively, and high purity (Scheme 3, Figure 1a). The first amino acid was anchored to a NALdehyde-1 or -2 polystyrene support by reductive amination. The second amino acid was coupled as the Fmoc-protected symmetrical anhydride, and further chain elongation proceeded using piperidine/DMF (1:4) for Fmoc removal and HBTU−HOBt for couplings. Upon completion of the sequence and Fmoc removal, treatment with TFA/DCM (50:50) released the peptide product with concomitant removal of *tert*-butyl protecting groups (Scheme 3).

Scheme 3. Solid-Phase Synthesis of H-Tyr-Gly-Gly-Phe-Leu-OH or H-Tyr-Gly-Gly-Phe-Leu-Pentyl-NH_2[a]



[a] Reaction conditions: (a) H-Leu-O*t*Bu·HCl or BocHN−$(CH_2)_5NH_2$·TsOH, $NaBH_3CN$, DMF, 24 h, rt; (b) Fmoc-Phe-OH or Fmoc-Leu-OH, DIPCDI, DCM/DMF 9:1, 16h, rt; (c) solid-phase synthesis with Fmoc/*t*Bu strategy; d) TFA/H_2O (95:5) or TFA/DCM (50:50), 2 h, rt. R_1, COO*t*Bu; R_2, $CH(CH_3)_2$; R_3, CH_2Ph. Or R_1, $(CH_2)_4NHBoc$; R_2, H; R_3, $CH(CH_3)_2$.

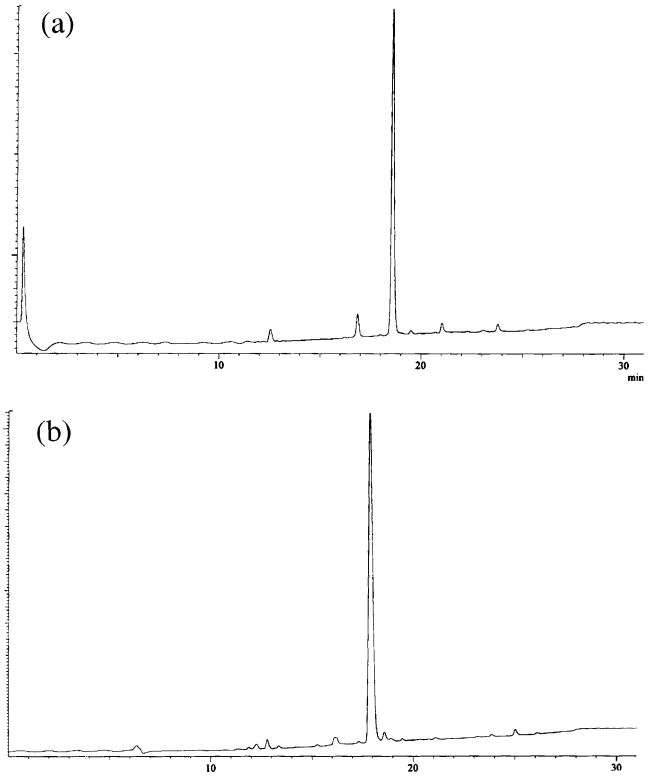


Figure 1. HPLC chromatogram (220-nm) of crude Leu-enkephalin **13** (a) and crude C-terminally modified Leu-enkephalin **14** (b), synthesized on NAL-2.

Attempts at synthesis of C-terminal peptide aldehydes released only traces of the desired product from the resin. This is in contrast to the efficient synthesis of peptide aldehydes on an *o*-BAL handle.[20] The poor performance of the NAL-2 handle in the synthesis of peptide aldehydes may be due to nucleophilic attack from the naphthalene core on the aldehyde (Friedel−Craft-type acylation), preventing release of the peptide aldehyde from the resin. In a second application, Leu-enkephalin C-terminally modified as a *N*-5-aminopentyl amide was synthesized on a NALdehyde-2 resin. This peptide was assembled smoothly on solid phase and isolated in 85% crude yield and high purity (Figure 1b).

Conclusion

We have developed and evaluated two new regioisomeric backbone amide linkers based on dialkoxynaphthalene aldehyde (NALdehyde) structures. Efficient and reliable reductive amination anchored the first building block, forming a NAL linkage. Using standard procedures for Fmoc-based solid-phase peptide synthesis, a series of peptides were prepared on the NAL handles. The final products were released from the support with TFA/H_2O (95:5) or TFA/DCM (50:50) in high crude yields and purity. NAL-2 was found to be more acid-labile under low-acid conditions, whereas NAL-1 was easily cleaved under high-acid conditions. The high acid-lability of NAL-1, in which only one of the alkoxy substituents provides resonance stabilization of the carbenium ion during cleavage, may be explained by a higher ground-state destabilization because of sterical repulsion with the alkoxy group peri to the anchoring point, leading to a more labile linkage to the peptide.[11] Both naphthalene linkers were more acid-stable than *o*-BAL and *p*-BAL handles. This lower lability may be useful in applications for which a more stable linkage to the solid-phase during synthesis is required. In addition, the different steric environments around the linkage site may make them useful for applications for which steric interactions are an

issue. Further modifications of the naphthalene aldehyde core structure for the construction of new handles are in progress.

Experimental Section

General Remarks. High-loading PS resin, all amino acids, and HBTU were obtained from NovaBiochem, and HOBt was from Quantum Richelieu. Solid-phase reactions were performed in polypropylene syringes equipped with a polyethylene filter placed on a shaker. Melting points were measured in open capillary tubes. Merck TLC Aluminum Sheets Silica Gel 60 F_{254} plates were used for TLC. Spots were visualized by UV light. Analytical HPLC was performed on a Waters system equipped with a 600 pump and a 996 PDA detector on a Waters Symmetry C18 (3.9 × 50 mm, 4 μm, 60 Å) column, running a 1 mL/min liniar gradient 0−95% buffer B over 15 min (buffer A, 0.1% TFA in H_2O; buffer B, 0.1% TFA in CH_3CN). HPLC/MS analysis was performed on a Shimadzu 2010, using a Phenomenex Jupiter C5 column (5 μm, 300 Å) and a 1 mL/min linear gradient from 3 to 95% buffer B over 18 min (buffer A, 0.025% TFA in H_2O; buffer B, 0.025% TFA in 90% aq CH_3CN). ^{1}H NMR spectra were recorded at 300 MHz on a Varian Mercury 300 or at 500 MHz on a Varian Inova 500 spectrometer. AAA was performed with Waters pico-tag in duplicate after hydrolyzing resin samples for 15 h at 130 °C with concentrated HCl/propionic acid (1:1). ESI-MS was performed on a Micromass LCT mass spectrometer.

5-(8-Formyl-5-methoxynaphthalene-1-yl-oxy)-pentanoic Acid Ethyl Ester (5). 8-Hydroxy-4-methoxynaphthalene-1-aldehyde[13,14] (2.0 g, 9.9 mmol) was dissolved in dry DMF (30 mL), and KO*t*Bu (1.2 g, 10.9 mmol) was added, followed by ethyl 5-bromovalerate (1.7 mL, 10.9 mmol), and the mixture was stirred overnight at 60 °C. The mixture was slowly poured into ice water (150 mL) while stirring. The product was collected by filtration, and recrystallized from EtOH. Yield, 2.6 g (80%); mp 92−93 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.07 (s, 1H, CHO), 8.05 (d, J = 8.1 Hz, 1H, ArH), 7.95 (d, J = 8.4 Hz, 1H, ArH), 7.42 (t, J = 8.1 Hz, 1H, ArH), 7.01 (d, J = 7.7 Hz, 1H, ArH), 6.89 (d, J = 8.1 Hz, 1H, ArH), 4.19 (t, J = 6.3 Hz, 2H, Ar−O−CH_2), 4.13 (q, J = 7.1 Hz, 2H, O−CH_2), 4.05 (s, 3H, Ar−O−CH_3), 2.41 (t, J = 7.1 Hz, 2H, CH_2CO), 2.0−1.92 (m, 2H, CH_2), 1.90−1.79 (m, 2H, CH_2), 1.25 (t, J = 7.1 Hz, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 194.2 (*C*HO), 173.2 (*C*OOEt), 159.4 (*C*4), 155.6 (*C*8), 129.3 (*C*2), 127.8 (*C*10), 127.1 (*C*6), 125.8 (*C*9), 124.5 (*C*1), 115.3 (*C*5), 108.5 (*C*7), 103.9 (*C*3), 68.6 (O*C*H_2), 60.4 (COO*C*H_2), 55.9 (O*C*H_3), 33.8 (*C*H_2COOEt), 28.7 (OCH$_2$*C*H_2), 21.7 (*C*H_2CH$_2$COOEt), 14.2 (*C*H_3). Anal. Calcd for $C_{19}H_{22}O_5$ (330.41): C, 69.06; H, 6.72. Found: C, 68.80; H, 6.75.

5-(8-Formyl-5-methoxynaphthalene-1-yl-oxy)-pentanoic Acid (6). Compound **5** (2.0 g, 6.0 mmol) was dissolved in THF (15 mL), and 1 M aq NaOH (18 mL) was added. After stirring for 18 h, the mixture was acidified by addition of 6 M HCl (8 mL), followed by addition of ice to facilitate precipitation. The product was collected by filtration and dried. Yield, 1.8 g (quantitative); mp 185−187 °C; ^{1}H NMR (500 MHz, [d_6] DMSO) δ 12.02 (br s, 1H, COOH), 10.92 (s, 1H, CHO), 7.87 (d, J = 8.2 Hz, 1H, ArH), 7.83 (d, J = 8.5 Hz, 1H, ArH), 7.48 (t, J = 8.2 Hz, 1H, ArH), 7.20 (d, J = 7.6 Hz, 1H, ArH), 7.10 (d, J = 8.2 Hz, 1H, ArH), 4.18 (t, J = 6.4 Hz, 2H, Ar−O−CH_2), 4.01 (s, 3H, CH_3), 2.29 (t, J = 7.3 Hz, 2H, CH_2CO), 1.90−1.77 (m, 2H, CH_2), 1.73−1.55 (m, 2H, CH_2); ^{13}C NMR (300 MHz, [d_6] DMSO) δ 194.0 (*C*HO), 174.9 (*C*OOH), 159.5 (*C*4), 155.8 (*C*8), 129.5 (*C*2), 128.0 (*C*10), 127.1 (*C*6), 127.0 (*C*1), 124.5 (*C*9), 115.2 (*C*5), 109.7 (*C*7), 105.3 (*C*3), 69.1 (O*C*H_2), 56.9 (O*C*H_3), 34.0 (*C*H_2COOH), 28.8 (OCH$_2$*C*H_2), 22.0 (*C*H_2CH$_2$COOH), Anal. Calcd for $C_{17}H_{18}O_5$ (302.12): C, 67.54; H, 6.00. Found: C, 67.27; H, 6.06. HR-MS (FAB+) Calcd for $C_{17}H_{18}O_5$ (monoisotopic), 303.1232; found, 303.1272 $[MH]^+$.

2,7-Dimethoxynaphthalene (8). In a round-bottomed flask equipped with a heavy stirring bar, K_2CO_3 (52 g, 375 mmol) was suspended in DMF (120 mL), and the mixture was degassed with argon. 2.7-Dihydroxynaphthalene (15 g, 93 mmol) was added, followed by CH_3I (29 mL, 468 mmol), and the mixture was stirred overnight at 50 °C. The dark brown mixture was poured into water (300 mL) and extracted with DCM (2 × 200 mL). The organic layer was backwashed with water (2 × 400 mL), dried (Na_2SO_4), and evaporated in vacuo. The brown residue was recrystallized from MeOH, furnishing the product as a slightly tanned powder. Yield, 11.7 g (66%); mp 138−139 °C (lit. 138 °C);[15] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 (d, J = 9.0 Hz, 2H, ArH), 7.08 (d, J = 2.1 Hz, 2H, ArH), 7.02 (dd, ^3J = 9.0 Hz, ^4J = 2.6 Hz, 2H, ArH), 3.93 (s, 6H, OCH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 158.5 (*C*2 + *C*7), 136.2 (*C*9), 129.4 (*C*4 + *C*5), 124.5 (*C*10), 116.2 (*C*3 +*C*6), 105.6 (*C*1 + *C*8), 55.5 (O*C*H_3). Anal. Calcd for $C_{12}H_{12}O_2$ (188.24): C, 76.56; H, 6.44. Found: C, 76.56; H, 6.50.

2,7-Dimethoxynaphthalene-1-aldehyde (9). Compound **8** (8.7 g, 46.2 mmol) was suspended in dry DMF (20 mL), $POCl_3$ (4.8 mL, 52 mmol) was added, and the suspension was stirred overnight at 60 °C, forming a clear brown solution. Ice water (200 mL) was added, and the mixture was stirred overnight at room temperature. The aldehyde was removed by filtration and recrystallized from MeOH. Yield, 7.8 g (78%); mp 101−102 °C (lit. 101 °C);[15] ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.90 (s, 1H, CHO), 8.85 (d, J = 2.6 Hz, 1H, ArH), 7.98 (d, J = 9.0 Hz, 1H, ArH), 7.66 (d, J = 9.0 Hz, 1H, ArH), 7.12 (d, J = 9.0 Hz, 1H, ArH), 7.07 (dd, ^3J = 9.0 Hz, ^4J = 2.6 Hz, 1H, ArH), 4.08 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 192.2 (*C*HO), 164.8 (*C*2), 161.5 (*C*7), 137.5 (*C*4), 133.5 (*C*9), 129.9 (*C*5), 124.0 (*C*10), 117.6 (*C*6), 115.7(*C*3), 109.7 (*C*1), 103.7 (*C*8), 56.7 (C2−O*C*H_3), 55.7 (C7−O*C*H_3); Anal. Calcd for $C_{13}H_{12}O_3$ (216.25): C, 72.20; H, 5.60. Found: C, 71.60; H, 5.41.

2-Hydroxy-7-methoxynaphthalene-1-aldehyde (10). Compound **9** (2.0 g, 9.3 mmol) was dissolved in dry DCM (15 mL), aluminum chloride (3.7 g, 27.8 mmol) was slowly added, and the dark red solution was stirred overnight at room temperature. The mixture was cautiously transferred to a separation funnel containing brine (50 mL) and extracted with ethyl acetate (2 × 60 mL), and the organic layer was backwashed with brine (2 × 50 mL), dried (Na_2SO_4), and evaporated in vacuo. Colored impurities were removed on a short silicagel column (ethyl acetate/hexane 1:1), furnishing the product as yellow crystals. Yield, 1.5 g (80%); mp 126−

128 °C (lit. 130 °C);[15] ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.75 (s, 1H, CHO), 7.89 (d, J = 9.0 Hz, 1H, ArH), 7.68 (m, 2H, ArH), 7.07 (dd, ^3J = 9.0 Hz, ^4J = 2.6 Hz, 1H, ArH), 6.97 (d, J = 9.4 Hz, 1H, ArH), 3.96 (s, 3H, OCH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 193.0 (*C*HO), 165.8 (*C*2), 160.6 (*C*7), 139.2 (*C*4), 135.1 (*C*9), 131.3 (*C*5), 123.3 (*C*10), 116.5 (*C*6), 115.7 (*C*3), 111.1(*C*1), 99.4 (*C*8), 55.7 (O*C*H$_3$). Anal. calcd for $C_{12}H_{10}O_3$ (202.22): C, 71.27; H, 4.99. Found: C, 71.00; H 4.81.

5-(1-Formyl-7-methoxynaphthalene-2-yl-oxy)-pentanoic Acid Ethyl Ester (11). Compound **10** (0.5 g, 2.5 mmol) was dissolved in DMF (5 mL), and KO*t*Bu (0.3 g, 2.8 mmol) was added, giving a dark brown solution. After 5 min, ethyl-5-bromvalerate (0.4 mL, 2.8 mmol) was added, and the mixture was stirred overnight at 60 °C. DMF was removed in vacuo, and the brown residue was run through a short silicagel column (hexanes/ethyl acetate 2:1) and triturated from hexane to yield the product as an off-white powder. Yield, 0.600 g (73%); mp 90−91 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.90 (s, 1H, CHO), 8.84 (d, J = 2.1 Hz, 1H, ArH), 7.95 (d, J = 9.0 Hz, 1H, ArH), 7.65 (d, J = 8.5 Hz, 1H, ArH), 7.07 (m, 2H, ArH), 4.25 (t, J = 6.0 Hz, 2H, Ar−OCH_2−), 4.14 (q, J = 7.3 Hz, 2H,OCH_2−C), 3.97 (s, 3H, Ar−OCH_3), 2.42 (t, J = 7.3 Hz, 2H, CH_2CO), 1.93 (m, 4H, CH_2), 1.27 (t, J = 6.4 Hz, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 192.2 (*C*HO), 173.4 (*C*OOEt), 164.5 (*C*2), 161.7 (*C*7), 137.5 (*C*4), 134.0 (*C*9), 129.9 (*C*5), 124.2 (*C*10), 117.6 (*C*6), 116.1 (*C*3), 110.5(*C*1), 103.7 (*C*8), 69.1 (ArO*C*H$_2$), 60.6 (COO*C*H$_2$), 55.7 (O*C*H$_3$), 34.1 (*C*H$_2$COOEt), 29.0 (OCH$_2$*C*H$_2$), 21.9 (*C*H$_2$CH$_2$COOEt), 14.5 (*C*H$_3$). Anal. Calcd for $C_{19}H_{22}O_5$ (330.41): C, 69.06; H, 6.72. Found: C, 68.95; H, 6.73.

5-(1-Formyl-7-methoxynaphthalene-2-yl-oxy)-pentanoic Acid (12). Compound **11** (0.9 g, 2.7 mmol) was dissolved in THF (16 mL), 0.5 M aqueous NaOH (16 mL, 8 mmol) was added, and the mixture was stirred for 2 h at rt. 1 M hydrochloric acid (16.0 mL, 16.0 mmol) was added, the aqueous layer was extracted with ethyl acetate (2 × 30 mL), and the organic layer was dried (Na_2SO_4) and evaporated in vacuo. The residue was triturated from hexane, and the product was obtained as an off-white powder. Yield, 0.62 g (76%); mp 156−159 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.02 (br s, 1H, COOH), 10.77 (s, 1H, CHO), 8.68 (d, J = 2.7 Hz, 1H, ArH), 8.16 (d, J = 8.8 Hz, 1H, ArH), 7.83 (d, J = 8.8 Hz, 1H, ArH), 7.35 (d, J = 9.1 Hz, 1H, ArH), 7.1 (dd, ^3J = 8.8 Hz, ^4J = 2.7 Hz, 1H, ArH), 4.27 (t, J = 6.1 Hz, 2H, Ar−OCH_2−), 3.86 (s, 3H, Ar−OCH_3), 2.31 (t, J = 7.3 Hz, 2H, CH_2CO), 1.83 (m, 2H, CH_2), 1.71 (m, 2H, CH_2); ^{13}C NMR (300 MHz, $CDCl_3$) δ 191.7 (*C*HO), 175.0 (*C*OOH), 165.1 (*C*2), 161.5 (*C*7), 138.5 (*C*4), 133.3 (*C*9), 130.9 (*C*5), 124.2 (*C*10), 117.0 (*C*6), 115.4 (*C*3), 112.2(*C*1), 103.9 (*C*8), 69.6 (O*C*H$_2$), 55.8 (O*C*H$_3$), 33.9 (*C*H$_2$COOH), 28.9 (OCH$_2$*C*H$_2$), 21.9 (*C*H$_2$CH$_2$COOH). Anal. Calcd for $C_{17}H_{18}O_5$ (302.12): C, 67.54; H, 6.00. Found: C, 67.01; H, 6.08. HR-MS (FAB$^+$) calcd for $C_{17}H_{19}O_5$ (monoisotopic): 303.1232. Found: 303.1249 $[MH]^+$.

Procedure for Anchoring NALdehydes to PS Resin. NALdehyde-1 or -2 (0.900 g, 3.0 mmol) and PyBOP (1.60 g, 3.0 mmol) were suspended in DMF (6 mL), DIEA (1.6 mL, 9.0 mmol) was added, and the mixture was stirred for 10 min at rt. The mixture was transferred to a filter syringe containing high-loading aminomethylated polystyrene resin (1.00 g, 1.00 mmol). The mixture was shaken overnight at rt. The derivatized resin was washed with DMF (10×), with DCM (5×), and MeOH and then air-dried.

H-Tyr-Gly-Gly-Phe-Leu-OH (13). NALdehyde-2-PS resin (0.250 g, 0.103 mmol), $NaBH_3CN$ (0.070 g, 1.030 mmol), and H-Leu-O*t*Bu·HCl (0.230 g, 1.030 mmol) in a filter syringe were suspended in DMF (5 mL) and shaken for 16 h, and the resin was washed with DMF (10×), DCM (5×). The resin was allowed to swell in DCM/DMF (9:1, 3 mL), Fmoc-Phe-OH (0.400 g, 1.030 mmol) and DIPCDI (0.081 mL, 0.515 mmol) were added, and the suspension was shaken. After 16 h, the resin was washed with DMF (10×) and DCM (5×). Fmoc removal was carried out with piperidine/DMF (1:4) for 5 min + 30 min, followed by washing of the resin with DMF (10×) and DCM (5×). Part of the resin (0.100 g) was taken into a filter syringe and subjected to further derivatization. Coupling reactions were performed as follows: Fmoc-AA-OH (4 equiv) was dissolved in DMF (5 mL), preactivated with HBTU (3.8 equiv), HOBt (4 equiv), and DIEA (7.8 equiv) for 5 min, and transferred to a filter syringe with the derivatized resin (coupling time, 1 h). After completion of the synthesis, the peptide was cleaved from the resin along with removal of the *tert*-butyl protecting groups with TFA/DCM (1:1, 1 mL) for 2 h. The resin was washed with TFA/DCM (1:1, 1 mL), and the combined solutions were evaporated in vacuo. The residue was suspended in diethyl ether, and after removal of the supernatant, the peptide was obtained as a white powder. Yield (crude), 13 mg (56%). HPLC data: t_R, 18.63 min. MS (ES$^+$), calcd $C_{28}H_{37}N_5O_7$: 555.6. Found: *m*/*z* 556.4 $[MH]^+$.

H-Tyr-Gly-Gly-Phe-Leu-pentyl-NH$_2$ (14). NALdehyde-2-PS resin (0.250 g, 0.103 mmol), $NaBH_3CN$ (0.070 g, 1.030 mmol), and BocHN$(CH_2)_5NH_2$·TsOH (0.230 g, 1.030 mmol) in a filter syringe were suspended in DMF (5 mL) and shaken for 16 h, and the resin was washed with DMF (10×) and DCM (5×). The resin was allowed to swell in DCM/DMF (9:1, 3 mL), Fmoc-Phe-OH (0.400 g, 1.030 mmol) and DIPCDI (0.081 mL, 0.515 mmol) were added, and the suspension was shaken. After 16 h, the resin was washed with DMF (10×) and DCM (5×). Fmoc removal was carried out with piperidine/DMF (1:4) for 5 + 30 min, followed by washing of the resin with DMF (10×) and DCM (5×). The resin (0.100 g) was taken in a filter syringe and subjected to further derivatization. SPPS and release from the resin with following workup were performed as described for H-Tyr-Gly-Gly-Phe-Leu-OH. The peptide was obtained as a white powder. Yield (crude), 22 mg (85%). HPLC data: t_R, 17.84 min. MS (ES$^+$), calcd $C_{33}H_{49}N_7O_6$: 639.9. Found: *m*/*z* 640.3 $[MH]^+$.

Reductive Amination (Table 1). The NALdehyde-derivatized resin (0.050 g, 0.050 mmol), $NaBH_3CN$ (0.031 g, 0.50 mmol), and H-Ala-OMe·HCl (0.070 g, 0.50 mmol) were suspended in a filter syringe with DMF (1.5 mL). After 24 h, the resin was washed with DMF (10×), DCM (5×), and MeOH before air-drying.

Acylation of Secondary Amine (Table 2). N^{α}-Fmoc-Gly-OH (0.15 g, 0.50 mmol) was dissolved in DCM/DMF (9:1, 3 mL) and transferred into a filter syringe containing the C-terminal protected amino acid derivatized resin (0.050 g, 0.050 mmol) to which DIPCDI (39 μL, 0.25 mmol) was added. The resin was shaken either for 1 h or overnight, followed by washing with DMF (10×), DCM (5×), and MeOH before air-drying.

Acidolytic Cleavage of Fmoc-Gly-Ala-OMe from the Resin (Table 3). The derivatized resin (0.010 g, 0.010 mmol) in a filter syringe was treated with TFA/water (95:5, 1 mL) or TFA/DCM (50:50, 1 mL) for 2 h. The supernatant was collected by suction filtration, and the resin was washed with TFA (2 mL). The combined solutions were concentrated in vacuo. The residue was redissolved in MeCN (1 mL), and cleavage yields were determined by HPLC−UV.

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