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Thiophene Backbone Amide Linkers, a New Class of Easily Prepared and Highly Acid-Labile Linkers for Solid-Phase Synthesis†

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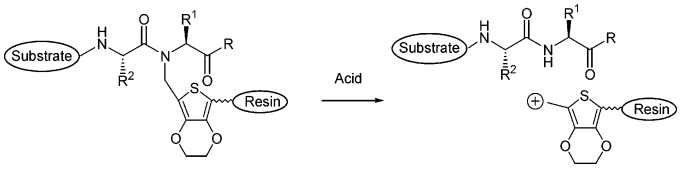

Solid-phase synthesis is of tremendous importance for small-molecule and biopolymer synthesis. Linkers (handles) that release amide-containing products after completion of solid-phase synthesis are widely used. Here we present a new class of highly acid-labile backbone amide linkers (BAL handles) based on 3,4-ethylenedioxythiophene (EDOT), which we have termed T-BAL. These thiophene linkers are synthesized in three convenient steps from commercially available EDOT. In the linker design, the spacer was introduced to the EDOT core either via a carbon−carbon bond or via a thioether linkage. Introduction of the spacer via a C−C bond was performed by a chemoselective Negishi coupling without transient protection of the aldehyde group to provide the T-BAL1 handle. Introduction via a thioether linkage was performed by a facile nucleophilic aromatic substitution between the brominated EDOT aldehyde and unprotected mercapto acids to provide T-BAL2 and T-BAL3 handles. The minimal use of protecting groups gave the corresponding linker molecules in few synthetic steps and in good yields. After anchoring of the linker to a polymeric support, introduction of the first amino acid was achieved by reductive amination, giving a secondary amine. A following acylation of the secondary amine with a symmetrical amino acid anhydride resulted in a backbone amide linkage between the handle and the growing substrate (e.g., peptide chain). After solid-phase synthesis, the substrates could be released from the resin by either low acid conditions using 1% TFA in CH_2Cl_2 or high acid conditions such as 50% TFA in CH_2Cl_2. Peptide thioesters could be released from the T-BAL1 handle under very mild conditions using aqueous acetic acid. *Tert*-butyl based protecting groups, *tert*-butyl esters, *tert*-butyl ethers, and Boc groups, as well as dimethyl acetals were relatively stable to these mild conditions for release of the peptides.

Introduction

The backbone amide linker (BAL) methodology has since the first reports in the mid-1990s become a widely used tool in solid-phase synthesis.[1,2] In the BAL approach, the growing peptide chain is generally anchored through a backbone amide, giving easy access to cyclic and C-terminal modified peptides, e.g., peptide aldehydes (Figure 1).

The BAL approach has furthermore been extended to the synthesis of a large variety of amide containing small molecules, oligosaccharides, and various amine substrates.[3] The BAL concept was first implemented in a tris(alkoxy)benzyl system, which allowed release of final products by treatment with

† Part of this work has been presented in preliminary form in Boas, U.; Jessing, M.; Christensen, J. B.; Jensen, K. J. *Proceedings of the 18th American Peptide Symposium*, Boston, 2003; pp 118−120. Boas, U.; Brask, J.; Christensen, J. B.; Jensen, K. J. *Proceedings of the 27th European Peptide Symposium*, Sorrento, 2002; pp 50−51. Boas, U. Ph.D. Thesis, University of Copenhagen, 2002.

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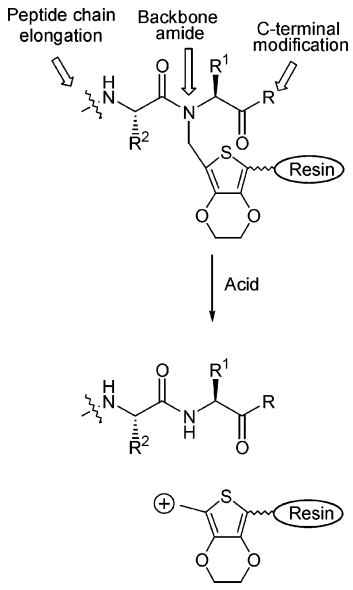


FIGURE 1. Schematic depiction of the solid-phase synthesis and release of peptides using EDOT-based BAL handles.

concentrated trifluoroacetic acid (TFA).[1,2] Later analogues, such as mono-[4] and dialkoxybenzylaldehydes,[5] indol, and[6] and naphthalene-based BAL handles, are somewhat less acid-labile in the release of peptide substrates.[7,8]

The thiophene backbone amide linkers (T-BAL1, T-BAL2, T-BAL3) are a new set of handles based on the 3,4-ethylenedioxythiophene (EDOT) core structure. EDOT has found wide use as an electron-donating monomer in conducting materials.[9] The electron-richness of EDOT should make it very suitable as carbenium ion stabilizing core in acid-labile linkers relying on the backbone amide linker principle (Figure 2).

In the final handle design all four positions on the thiophene ring are substituted. The spacer is an alkyl moiety attached directly to the thiophene ring via a carbon−carbon bond, or via a thioether linkage. The introduction of a sulfur atom on the EDOT should provide higher acid lability by additional stabilization of the carbenium ion (Figure 2).

Results and Discussion

Synthesis of T-BAL1. In the synthetic sequence, EDOT was first formylated, to give a more stable (less electron-rich) and crystalline building block (Scheme 1). The aldehyde **1** was obtained in 71% yield by Vilsmeier−Haack formylation on EDOT at −10 °C by slow addition of $POCl_3$, using DMF as both reactant and solvent.[10] Iodination of **1** went smoothly in 84% yield at room temperature. Using *N*-iodosuccinimide (NIS) activated by acetic acid,[11,12] no oxidation of the aldehyde moiety

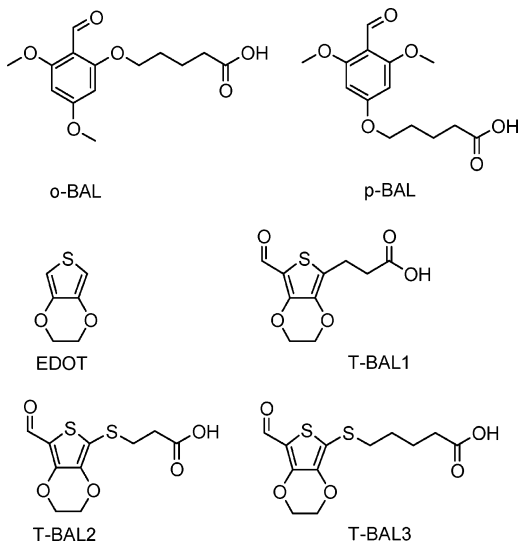
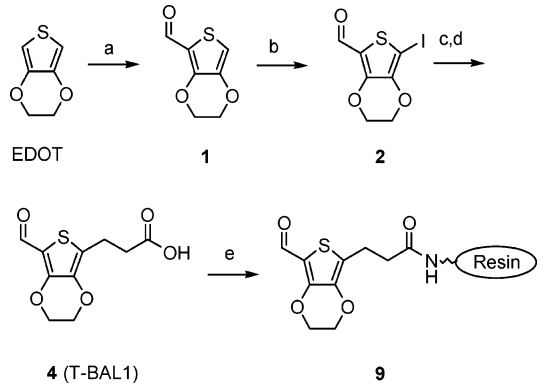


FIGURE 2.

SCHEME 1[a]



[a] Reagents and conditions: (a) $POCl_3$, DMF, −10 °C, 30 min, 72%; (b) NIS, AcOH, $CHCl_3$, 25 °C, 16 h or NCS, NaI, acetone, 25 °C, 16 h, 85%; (c) $IZn(CH_2)_2COOMe$, $Pd_2(dba)_3$, TFP, DMF, 40 °C, 2 h; (d) LiOH (aq), THF, 25 °C, 2 h, 61%, two steps; (e) HBTU, HOBt, DIPEA, alanine-derivatized polystyrene resin, rt, 2 h, then capping with Ac_2O for 2 h.

was observed under the reaction conditions (Scheme 1).[13] Alternatively, the α-iodination was carried out with in situ generated NIS, from *N*-chlorosuccinimide (NCS) and NaI,[14] giving similar yield and purity of the product **2**.

Subsequently the spacer was introduced to the EDOT core by a Negishi C−C cross-coupling reaction between the alkylzinc reagent of methyl 3-iodopropanoate and **2**. DMF was used as solvent as this solvent generally gives better conversion yields with acid-derived organozinc reagents.[15] (3-Carboxymethylpropyl)-zinc-iodide was preferred to longer alkyl esters, as a

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(11) Boas, U.; Dhanabalan, A.; Greve, D. R.; Meijer, E. W. *Synlett* **2001**, 634−636.
(12) For Activation of NBS with acetic acid, see: Wu, R.; Schumm, J. S.; Pearson, D. L.; Tour, J. M. *J. Org. Chem.* **1996**, *61*, 6906−6921.
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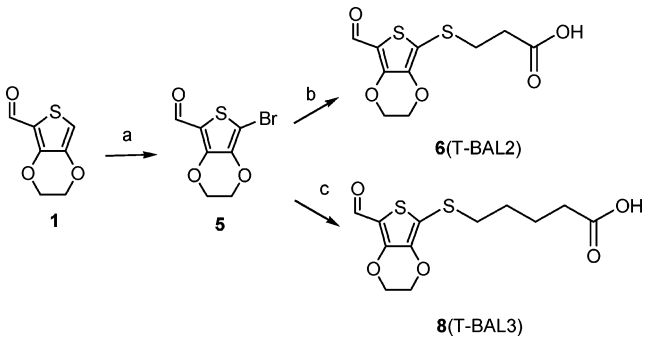
SCHEME 2 [a]



[a] Reagents and conditions: (a) NBS, CH_3CN, rt, 60 h, 91%; (b) 3-mercapto propionic acid, KOH, EtOH, 60 °C, 4 h, then citric acid (aq), 87%; (c) 5-mercapto pentanoic acid, KOH, EtOH, 60 °C, 5 h, then citric acid (aq), 85%.

result of higher reactivity of the corresponding zinc compound.[15] Applying tri-2-furylphosphine (TFP) as ligand for Pd has previously been shown to increase reaction rates and yields of the Negishi reaction.[16] Finally, 4 equiv of TFP was used together with 1 equiv of $Pd_2(dba)_3$ complex in order to create the highly reactive $Pd^0(TFP)_2$ catalyst during the process.[15] In this way a new carbon−carbon bond was formed without affecting either the unprotected aldehyde or the ethyl ester moiety. The intermediate ester was not isolated, to avoid separation problems. Instead the ester was hydrolyzed subsequently under mild conditions by aqueous LiOH, in a one-pot reaction yielding the carboxylic acid, which could be purified from remaining iodoaldehyde **2** by extraction. The one-pot reaction afforded the pure linker **4** in 61% yield as a crystalline compound, stable to air and light, (Scheme 1).

Synthesis of T-BAL2 and T-BAL3. With the goal of further simplifying the synthesis of the handle and increase its acid-lability, we introduced an exo-cyclic sulfur at the connection with the spacer. Initial studies on the nucleophilic substitution on the thiophene α-position with mercaptoalkyl-carboxylic acids revealed that α-brominated thiophene gave higher yields and reaction rates than the corresponding iodide, as deiodination of the thiophene became the prominent reaction. The corresponding bromide (**5**, Scheme 2) was synthesized from EDOT aldehyde **1** in 85% yield using NBS in CH_3CN at room temperature.

Nucleophilic aromatic substitution for introduction of the spacer moiety was initially conducted as a model experiment, between **5** and 1-hexanethiol. This reaction was fast, and TLC showed complete conversion to the corresponding product, *S*-((5-formyl-3,4-ethylenedioxy) thiophene-2-yl)-1-thiohexane, after 30 min at room temperature.

In contrast, the substitution with the mercaptoalkyl-carboxylic acids proceeded slowly at room temperature; however, by raising the temperature to 60 °C the reactions took place at a satisfactory rate. The corresponding thiophene carboxylate adducts were acidified under mild conditions using dilute citric acid to the corresponding carboxylic acids **6** and **8** in 87% and 85% yields, respectively (Scheme 2).

Although 3-mercaptopropionic acid is commercially available, 5-mercaptopentanoic acid (**7**) was synthesized from the corresponding bromovaleric acid methyl ester and thiourea, similar

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TABLE 1. Comparison of Cleavage Yields for Leu-Enkephalin on T-BAL1, *o*-BAL, and *p*-BAL[a]

entry	cleavage conditions	T-BAL1 (AAA, %)	*o*-BAL (AAA, %)[19]	*p*-BAL (AAA, %)[19]
1[a]	95% TFA (aq), 2 h	66	87	89
2[a]	50% TFA (CH_2Cl_2), 2 h	76	82[b]	83[b]
3[a]	5% TFA (CH_2Cl_2), 2 h	71	71[b]	70[b]
4[a]	1% TFA (CH_2Cl_2), 2 h	84	70	64

[a] For T-BAL1 a resin with a loading of 0.36 mmol/g was used, whereas for *o*-/*p*-BAL a resin with a loading of 0.40 mmol/g was used. [b] Cleavage was conducted over 15 min.

to the procedure by Jung and co-workers.[17] Coupling of the linker molecules to the amino functionalized resin by amide bond formation was achieved by (benzotriazol-1-ylozy)tris-(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) or *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methyl-methanaminium hexafluorophosphate (HBTU) as coupling agent and DIPEA as base.

Solid-Phase Evaluation of T-BAL1. After initial anchoring to the solid phase, polystyrene, the T-BAL1-derivatized resin was applied for the synthesis of unprotected and protected peptides. Furthermore, it was used for the synthesis of C-terminal thioesters and ketene dithioacetals. In all cases, the first building block was anchored by reductive amination. However, at first this reaction did not go to completion, and different solvent systems were tried in the anchoring of the first building block. From the solvent systems used, THF/MeOH/TMOF (4:1:5) proved to give the highest yield of attached amine to the T-BAL1 handle for amino acids and primary amines in general.

Then, solid-phase peptide synthesis was conducted under standard fluorenyloxycarbonyl (Fmoc) conditions, and subsequently the peptides were released with different acidic cleavage mixtures containing TFA, trifluoromethanesulfonic acid (TFMSA),[18] or AcOH. After 2 h cleavage time the cleavage mixtures and residual resins were analyzed with HPLC, amino acid analysis (AAA)/ and magic angle spinning (MAS) NMR.

The immunoreactive pentapeptide Leu-Enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH) was chosen as model substrate, as this peptide has been applied in earlier studies concerning development of BAL handles and thus provides comparative information. In the solid-phase synthesis of Leu-Enkephalin, cleavage yields determined by AAA gave comparable yields for the T-BAL1 handle and the *o*- and *p*-BAL handles under high acid conditions. However, somewhat better cleavage yields were obtained for T-BAL1 at low acid concentrations (Table 1). Indications for initial high-yield release of peptides from the T-BAL1 resin under mild conditions were the low amounts of peptide released in a second acidolytic release step by treatment of the peptidyl-resin with TFA/CH_2Cl_2 (1:1) for 24 h. Similarly, only small amounts of peptide were released when performing the second acidolysis with the stronger acid TFMSA.

MAS NMR on the peptidyl-resin (data not shown) revealed a very low content of Leu-Enkephalin present on the resin after acidolytic treatment with 1% TFA in CH_2Cl_2, also indicating a high acid-lability under these conditions for release of the peptide.

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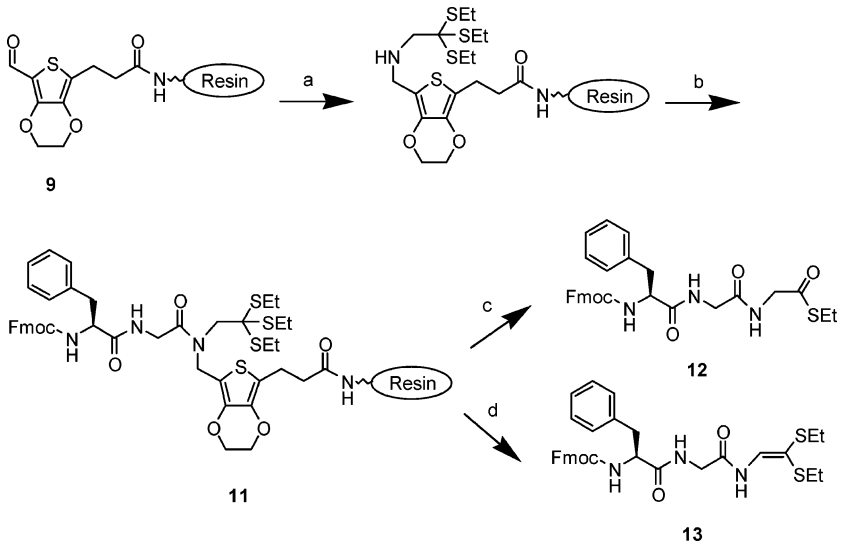
(18) No cleavage yields are reported for cleavage with TFMSA as they did not give higher yields than for similar concentrations of TFA.

SCHEME 3 [a]



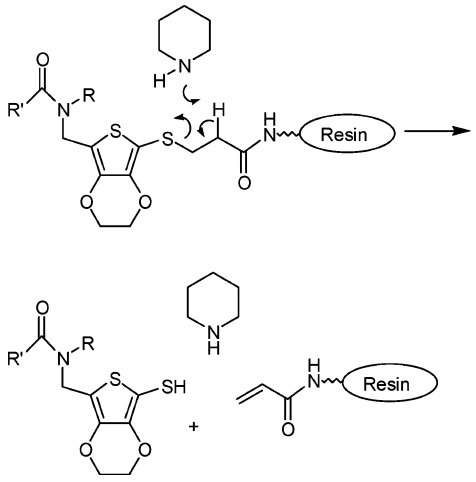
[a] Reagents and conditions: (a) $^{-}Cl^{+}H_3N\text{-}CH_2\text{-}C(EtS)_3$, $NaBH_3CN$, DMF, 16 h; (b) Fmoc-Gly-OH, DIPCDI, CH_2Cl_2/DMF 9:1, 16 h, then SPPS using Fmoc strategy; (c) TFA/H_2O (19:1), 2 h, 65% or HOAc/H_2O (1:1), 2 h, 40%; (d) TFA/CH_2Cl_2 (1:1), 2 h, 63%.

For further test of the methodology with several di- and tripeptides with different C-terminal functionalities, low-acid conditions combined with quenching of the acidic mixture with base released the protected peptides without affecting acid-labile protecting groups, e.g., the Boc- or the *tert*-butylester group. The possibility to cleave off peptides having a thioester group is intriguing as these activated substrates may be applied for subsequent synthesis of polypeptide or protein substrates by Kent segmental coupling strategy. A tripeptide trithioortho ester (**11**) was synthesized on the T-BAL1-derivatized solid phase, where the trithioortho ester group serves as a base-stable but acid-labile masking group for the C-terminal thioester and ketene dithioacetal functionalities. The trithioortho ester was cleaved from the resin in 63% yield either as the thioester (**12**) using 95% aqueous TFA or, again, in 63% yield as the ketene dithioacetal (**13**) using anhydrous conditions, e.g., TFA/CH_2Cl_2 (1:1) (Scheme 3).[20]

It is quite remarkable that peptide thioesters could also be released in 40% yield from T-BAL1 with 50% aqueous acetic acid. This hyper acid-lability of the T-BAL1 handle appears to be specific for the peptide trithioetho ester, in which steric congestion when attached to the resin facilitates the cleavage process.

The dipeptide aldehyde Fmoc-Lys-Gly-H was synthesized and released in 62% yield by TFA/CH_2Cl_2 (1:1). In contrast, when the peptide was released with 1% TFA in CH_2Cl_2, the acid-labile C-terminal dimethyl acetal, precursor of the aldehyde, mainly remained intact. Furthermore, we investigated the possibility to release more basic functionalities (e.g., amines) directly from the T-BAL1 handle.[21] We found that the aliphatic primary amine Fmoc-HN$(CH_2)_2$-NH_2 and the secondary amine FmocHN$(CH_2)_2$NHCH_2Ar could be released by TFA/CH_2Cl_2 (1:1) after 2 h at 60 °C in moderate yields of 27% and 37%, respectively.

SCHEME 4. Proposed Mechanism for β-Elimination Leading to Decreased Loading during Solid-Phase Synthesis on the T-BAL2 Handle



Solid-Phase Evaluation of T-BAL2 and T-BAL3. Upon performing solid-phase synthesis of Leu-Enkephalin on T-BAL2, decreasing yields of released peptide were observed after each coupling. An explanation for this observation may be the susceptibility of this linker to β-elimination when subjected to the basic conditions required for cleavage of the Fmoc group after each solid-phase coupling (Scheme 4). Because of this considerable drawback, solid-phase synthesis based on this linker was not investigated further.

To investigate the initial reductive amination and acylation steps in the synthesis of the peptide backbone, several dipeptide sequences were synthesized and released from the T-BAL3 linker. For the reductive amination step the solvent system TMOF/MeOH (4:1) resulted in significantly better reaction yields compared to 1% acetic acid in MeOH. TMOF is a well-known condensation reagent for the formation of imines. In addition, higher temperatures and longer reaction times led to increasing yields. Shorter reaction times and similar yields were

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TABLE 2. Reaction Conditions and Yields for the Reductive Amination of T-BAL3[a]

peptide	additives and solvents	reaction conditions	yield (%)
Fmoc-Ala-Gly-OH	AcOH/MeOH (1:99)	microwave, 60 °C, 10 min	71
Fmoc-Ala-Gly-OH	AcOH/MeOH (1:99)	microwave, 60 °C, 2 × 10 min	75
Fmoc Ala-Gly-OH	TMOF/MeOH (4:1)	microwave, 60 °C, 2 × 10 min	71
Fmoc Ala-Gly-OH	HOAc/TMOF/MeOH (1:80:20)	microwave, 60 °C, 2 × 10 min	68
Fmoc Ala-Gly-OH	1 equiv of $Sc(Otf)_3$, MeOH	microwave, 60 °C, 10 min	70
Fmoc Ala-Gly-OH	1 equiv of $Ti(O^iPr)_4$, MeOH	microwave, 60 °C, 10 min	69
Fmoc-Ala-Leu-OH	TMOF/MeOH (4:1)	microwave, 60 °C, 10 min	10
Fmoc-Ala-Leu-OH	1 equiv of $Sc(Otf)_3$, MeOH	microwave, 60 °C, 10 min	13
Fmoc-Ala-Leu-OH	1 equiv of $Ti(O^iPr)_4$, MeOH	microwave, 60 °C, 10 min	11
Fmoc-Ala-Leu-OH	TMOF/MeOH (4:1)	heater shaker, 60°C, 16 h	32
Fmoc-Ala-Leu-OH	TMOF/MeOH (4:1)	heater shaker, 100°C, 1 h	37

[a] Conditions for acylation of the secondary amine: Fmoc-Ala-OH (10 equiv) and DIPCDI (5 equiv) in CH_2Cl_2/DMF (9:1), rt., 2 h, repeated once; loading determined by Fmoc quantification.

TABLE 3. Cleavage (Release) Yields from H-Tyr-Gly-Gly-Phe-(T-BAL3-Ile-PS)LeuNH$_2$ Analyzed by Amino Acid Analysis Using Ile as Internal Reference Amino Acid (IRAA)[a]

entry	cleavage conditions	Leu-Ile ratio before	Phe-Ile ratio before	Leu-Ile ratio after	Phe-Ile ratio after	yield (%)
1	TFA/H_2O (95:5)	0.71	0.73	0.11	0.044	85−94
2	TFA/CH_2Cl_2 (5:95)	0.71	0.73	0.17	0.16	76−78
3	TFA/CH_2Cl_2 (1:99)	0.71	0.73	0.71	0.73	0

[a] Cleavage (release) yield is here defined as (before − after)/before × 100%, where "before" is the peptidyl-resin prior to acid treatment, and "after" is subsequent to acid treatment.

achieved using microwave heating (Table 2). As a general trend, the reductive amination yields decreased with increasing bulkiness of the C-terminal amino acid, where dipeptides containing a C-terminal leucine were released in low to moderate yields. Addition of 1 equiv. of a Lewis acid such as $Sc(OTf)_3$ or $Ti(OiPr)_4$ did not result in improved yields. Applying other additives such as the condensation reagents TMS-Cl or Ph_3PBr_2 did not increase the effectiveness of the preceding imine formation either.

Generally, the use of Tentagel resin resulted in overall better cleavage yields in comparison to a pure polystyrene resin.

Finally, upon finding the optimal conditions for the reductive amination and acylation steps, the effectiveness of the release of Leu-Enkephalin from the T-BAL3 handle was investigated by AAA. These investigations showed that high acid conditions (95% aqueous TFA) led to release of the peptide from T-BAL3 in excellent yields (85−94%). Low acid conditions (5% TFA in CH_2Cl_2) released the peptide in good yields (76−78%), whereas no release of peptide from the resin could be measured upon employment of a 1% TFA in CH_2Cl_2 cleavage mixture (Table 3).

Interestingly, release from the resin of the peptide H-Tyr-Gly-Gly-Phe-Leu-Gly-Gly-OH, which is less sterically congested around the anchoring point to the handle, resulted in somewhat lower yields of peptide. In this study the T-BAL1 and T-BAL3 had comparable acid-labilities, releasing 45−55% peptide under high acid conditions (95% TFA in CH_2Cl_2 or 95% aqueous TFA). In these investigations modest yields (8−12%) of peptide were released under low-acid conditions (1%TFA in CH_2Cl_2) from both handles. The lower yields in the release of this sequence, at 5% and 95% TFA, indicate that steric relief may play an important role in the release of the peptide from these thiophene based linkers. However, it was rewarding to observe that for this particular peptide, some release occurred with 1% TFA.

Conclusion

Three new BAL type handles based on the commercially available electron-rich building block EDOT have been prepared by a three step synthetic sequence. The new T-BAL handles have proven useful as a set of very acid-labile linkers utilized in the BAL solid-phase methodology. Generally, the new linkers are more acid-labile than the original trialkoxybenzyl based BAL handles. The new handles were used for synthesis of protected and partly protected peptides carrying acid-labile functionalities such as dimethyl acetals, *tert*-butyl esters, and *tert*-butyl ethers. Thioesters or ketene dithioacetals could, depending on the cleavage conditions be released from a T-BAL1 resin. The T-BAL handles are the first reported handles where peptide thioesters could be released under extremely mild conditions using aqueous acetic acid. This can possibly be rationalized by ground-state destabilization of the T-BAL1 anchored peptide due to the bulky trithioortho ester moiety. All of the peptides synthesized were released in high purity according to HPLC.

T-BAL2 and T-BAL3, which contain an exocyclic thioether as bridging point to the spacer, were synthesized via a more convenient route, well-suited for large scale synthesis compared to the T-BAL1 handle. However, upon performing solid-phase peptide synthesis (SPPS) on the T-BAL2 handle the yields of released peptide decreased rapidly during the course of the synthesis, most likely as a result of β-elimination. For the T-BAL3 handle, the bulkiness of the first amino acid building block had a large effect on the yield of the initial reductive amination, where Leu gave significantly lower yields. However, less bulky amino acids were incorporated in satisfactory yields. Investigations by AAA on the acid-lability of the T-BAL3 handle in the release of Leu-Enkephalin showed a somewhat lower acid-lability of T-BAL3 in comparison to the initial T-BAL1 handle. In addition, the release of a peptide less sterically congested around the linkage to T-BAL3 was studied, conforming in this case the effect of (lack of) steric bulk on release of peptide from the resin. Interestingly, this study revealed that steric factors (i.e. steric relief) during the release of the peptide may affect the yield of the released peptide, showing a tendency of lower release of less bulky peptides. However a more thorough study concerning the effect of sterically congested amino acids at the anchoring site will have to be performed, to show if the lower release yields are a common feature in the release of less sterically congested peptides from BAL-based handles. However, in general both T-BAL1 and T-BAL3 show somewhat higher acid-labilities in comparison to previously reported BAL handles based on a homo-aromatic core structures, making this novel class of

thiophene based linkers a promising motif for highly acid labile BAL handles.

Experimental Section

2-(3,4-Ethylenedioxythiophene) carbaldehyde (1).[23] 3,4-Ethylenedioxythiophene (50 g, 0.35 mmol) was dissolved in dry DMF (200 mL). The mixture was cooled to −10 °C (ice−ethanol bath) and $POCl_3$ (33 mL, 0.36 mmol) was added dropwise (15 min) in the cold. The mixture stirred 1 h at −10 °C, ice water (500 mL) was added and the mixture stirred overnight at room temperature. The aldehyde was filtered off, dissolved in CH_2Cl_2 (500 mL) and dried (Na_2SO_4). The CH_2Cl_2 filtrate was eluted through a short silica "plug" to remove colored byproducts, giving the dry product as slightly tanned crystals. Yield 42 g (71%); mp 145−146 °C (lit.[10] 142 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.91 (s, 1H), 6.80 (s, 1H), 4.37 (m, 2H), 4.28 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.7, 149.2, 142.5, 119.2, 111.4, 66.0, 65.1. Anal. Calcd ($C_7H_6SO_3$ (170.19)): C 49.40, H 3.56, S 18.84. Found: C 49.41, H 3.43 S 18.78. MS (FAB$^+$) found 170.96 (MH$^+$).

2-Iodo-(3,4-ethylenedioxythiophene) 5-carbaldehyde (2). Compound **1** (2.0 g, 11.8 mmol) was suspended in dry chloroform (10 mL), and glacial acetic acid (10 mL) was added followed by NIS (2.9 g, 13.0 mmol), giving a deep purple color of the mixture. The mixture was shielded from light and stirred overnight at room temperature, changing to a red suspension. Ethyl acetate (50 mL) was added and the organic layer was washed with 10% Na_2CO_3 (aq) (2 × 50 mL), $Na_2S_2O_3$ (sat.) (2 × 50 mL), and water (2 × 50 mL). The residue was dried (Na_2SO_4), evaporated in vacuo and recrystallized from ethanol to yield 2.9 g (84%) of the product as a bright yellow crystalline. Mp 156−157 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.78 (s, 1H), 4.36 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 1778.8, 147.0, 144.4, 123.4, 110.9, 65.4, 65.1. Anal. Calcd ($C_7H_5SO_3I$ (296.08)): C 28.39, H 1.71, S 10.83. Found: C 28.75, H 1.48 S 10.44. MS (FAB$^+$) found 296.83 (MH$^+$).

3-(5-Formyl-3,4-ethylenedioxy)thiophen-2-yl) Propionic Acid (4). Zinc dust (0.907 g, 13.6 mmol) was suspended in dry DMF (3 mL) and activated by addition of 1.2-dibromoethane (59 μL), followed by stirring 30 min at 60 °C, after cooling to room temperature. TMS−Cl (18 μL) was added and the mixture was stirred vigorously for 30 min at room temperature. The mixture was cooled on an ice bath and the iodoester **3**[24] (0.53 g, 2.3 mmol) was added dropwise in the cold; after 10 min the ice bath was removed. The mixture was stirred for 30 min at room temperature. generating the alkylzinc reagent (TLC (ethyl acetate) shows quenching of the iodide). The slurry was centrifuged and the supernatant was transferred to a mixture of compound **2** (0.50 g, 1.7 mmol), $Pd_2(dba)_3$ (15 mg, 0.014 mmol) and TFP (15 mg, 0.061 mmol) in dry DMF (2 mL); The mixture heats to approximately 40 °C spontaneously. The dark orange mixture was stirred for 2 h at 40 °C. HPLC showed quantitative conversion to the ester adduct. LiOH (0.71 g, 17.00 mmol in 12 mL water) was added, and the mixture stirred for 2 h at room temperature, hydrolyzing the ester. Water (100 mL) was added and the polar layer was extracted with CH_2Cl_2 (2 × 100 mL). Concentrated aqueous HCl (2.6 mL, 26 mmol) [25] was added and the water layer was quickly extracted with ethyl acetate (1 × 150 mL). The ethyl acetate layer was washed with brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was triturated from diethyl ether to give 0.250 g (61%) of the product as a curry tanned powder. Reprecipitation from ethyl acetate−hexane gave the product as yellow crystals. ^{1}H NMR (500 MHz, d_6-DMSO) δ 9.76 (s, 1H), 4.38 (m, 2H), 4.29 (m, 2H), 2.86 (t, ^3J = 7.3 Hz, 2H), 2.54 (t, ^3J = 7.3 Hz, 2H); ^{13}C NMR (125 MHz, d_6-DMSO) δ 179.5, 173.6, 149.5, 139.0, 129.3, 114.9, 66.2, 65.0, 34.0, 22.4. Anal. Calcd ($C_{10}H_{10}SO_5$ (242.26)): C 49.57, H 4.17, S 13.23. Found: C 49.10, H 4.08, S 12.98. MS (FAB$^+$) found 242.99 (MH$^+$).

2-Bromo-(3,4-ethylenedioxythiophene)-5-carbaldehyde (5). Compound **1** (4.04 g, 23.7 mmol) was suspended in dry acetonitrile (100 mL) and cooled to 0 °C. NBS (4.36 g, 26.0 mmol), 1.1 equiv, was added and the mixture was stirred for 60 h at room temperature, shielded from light and under nitrogen. The color changed from yellow to purple. The mixture was transferred with 150 mL ofethyl acetate to a separation funnel, washed with 10% aqueous Na_2CO_3 (2 × 200 mL), saturated $Na_2S_2O_3$ (2 × 200 mL) and water (2 × 200 mL), dried with $MgSO_4$, and evaporated in vacuo. Recrystallization twice from ethanol (60 mL) yielded 5.35 g (91%) of the bromide as yellow needles. Mp 138−140 °C (decomp); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.83 (s, 1H), 4.36 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.0, 147.9, 140.4, 118.8, 102.0, 65.5, 65.1. Anal. Calcd ($C_7H_5BrO_3S$ (249.03)): C 33.75, H 2.02, S 12.87. Found: C 33.48, H 1.79, S 13.98; MS (FAB$^+$) found 248.94 (M$^+$).

***S*-((5-Formyl-3,4-ethylenedioxy)thiophene-2-yl)-3-thiopropionic Acid (6).** KOH (1.14 g, 20.3 mmol), 3 equiv, and 3-mercaptopropionic acid (0.64 mL, 7.34 mmol), 1.2 equiv, were dissolved in ethanol (50 mL) and stirred for 10 min. Compound **5** (1.59 g, 6.38 mmol) was added initially giving a yellow solution that turned orange upon stirring under nitrogen for 4 h at 60 °C. CH_2Cl_2 (150 mL) was added and the mixture was extracted with water (150 mL) and saturated $Na_2S_2O_3$ (50 mL). The aqueous layer was backwashed with CH_2Cl_2 (100 mL) to remove residual bromide. The aqueous layer was acidified with citric acid (1.59 g, 7.57 mmol), and the solution was stirred for 10 min. CH_2Cl_2 (100 mL) was added and the mixture was stirred for another 40 min. The aqueous layer was extracted with additional CH_2Cl_2 (100 mL). The organic phase was dried with Na_2SO_4 and evaporated in vacuo. Yield 1.53 g (87%) as light yellow needles. Mp 109−113 °C (decomp); ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.0−10.2 (bs, 1H), 9.82 (s, 1H), 4.34 (m, 4H), 3.11 (t, ^3J = 7 Hz, 2H), 2.72 (t, ^3J = 7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.3, 177.0, 148.2, 142.7, 120.7, 119.1, 65.3, 64.9, 34.4, 30.9. Anal. Calcd ($C_{10}H_{10}O_5S_2$ (274.32)): C 43.78, H 3.67, S 23.38; found C 43.52, H 3.45, S 22.80. MS (FAB$^+$) found 273.97 (M$^+$) and 274.98 (MH$^+$).

5-Mercapto-pentanoic Acid (7). A mixture of thiourea (1.35 g, 17.7 mmol), 1.5 equiv, and 5-bromo-pentanoic acid ethyl ester (1.9 mL, 12.0 mmol) was refluxed in EtOH (25 mL) for 20 h. The solvent was removed in vacuo and 7.5 M NaOH (aq) (25 mL, 188 mmol), 15 equiv, was added. The mixture was stirred for an additional 16 h at 90 °C, under nitrogen. It was then cooled on an ice bath and 2M H_2SO_4 was added slowly under stirring. The organic product was extracted with CH_2Cl_2 (2 × 100 mL), dried with $MgSO_4$. Evaporation in vacuo gave a colorless oil in quantitative yield. The product was used at once in the next reaction as formation of disulfide was observed by prolonged storage, even under nitrogen.^{1}H NMR (300 MHz, $CDCl_3$) δ 11.3−10.2 (br. s, 1H), 2.54 (dt/q, $^3J_1$ = 7 Hz, $^3J_2$ = 8 Hz, 2H), 2.37 (t, ^3J = 7 Hz, 2H), 1.80−1.59 (m, 4H), 1.35 (t, ^3J = 7 Hz,1H).

***S*-((5-Formyl-3,4-ethylenedioxy)thiophene-2-yl)-5-thiopentanoic acid (8).** KOH (1.77 g, 31.5 mmol), 3 equiv, and freshly made 5-mercaptopentanoic acid **7** (1.42 g, 10.6 mmol), 1.0 equiv, were dissolved in 50 mL of ethanol and stirred for 10 min. Compound **5** (2.65 g, 10.6 mmol) was added initially giving a yellow solution. After stirring under nitrogen for 5 h at ∼ 60 °C, the solution had turned orange. Water (100 mL) and saturated $Na_2S_2O_3$ (50 mL) was added and the aqueous layer was washed with CH_2Cl_2 (2 × 120 mL). Citric acid (2.67 g, 12.7 mmol), 1.2 equiv, was added and the solution was stirred for 10 min. CH_2Cl_2 (2 × 100 mL) was added and the mixture stirred another 40 min before collection of the organic phase. The organic phase was

(22) Knochel, P.; Yeh, M. C. P.; Berk, S. C.; Talbert, J. *J. Org. Chem.* **1988**, *53*, 2390−2392.

(23) The correct IUPAC nomenclature for this compound is 2,3-dihydrothieno [3,4-*b*][1,4] dioxine-5-carbaldehyde, but for this and the following compounds the more brief "EDOT-nomenclature" will be applied.

(24) Tok, J. B. H.; Cho, J.; Rando, R. R. *Tetrahedron* **1999**, *55*, 5741−5758.

(25) Alternatively, 10% aqueous citric acid can be used in order to give a milder acidification of the reaction mixture.

backwashed with water (2 × 100 mL), dried with (Na_2SO_4) and evaporated in vacuo. Yield was 3.12 g (97%) of the product as a yellow powder containing a little disulfide. A part of the product, 2.13 g, was dissolved in MeOH (10 mL/g compound) and water was added (100 mL) giving a milky slurry. Upon standing overnight crystals precipitated out. The crystals were filtered with suction, washed with water and dried. Yield 1.80 g (85%) of light yellow needles. Mp 89−91 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.6−10.8 (bs, 1H, CO_2H), 9.77 (s, 1H, C*H*O), 4.34 (m, 4H, OCH_2CH_2O), 2.92 (t, ^3J = Hz, 2H), 2.38 (t, ^3J = 7 Hz, 2H), 1.75 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.0, 178.9, 148.2, 141.7, 122.9, 118.4, 65.3, 64.8, 35.8, 33.4, 28.8, 23.5. Anal. Calcd ($C_{12}H_{15}O_5S_2$ (302.37)): C 47.67, H 4.67, S 21.21. Found: C 48.02 H 4.61 S 21.39. MS (FAB$^+$) found 302.08 (M^+) and 303.08 (MH^+).

Anchoring T-BAL1 to Aminomethylated PS Resin via Ala as Internal Reference Amino Acid (IRAA) (9). Aminomethylated polystyrene resin (1.929 g, 0.694 mmol) was washed with DMF (3 × 10 mL) and CH_2Cl_2 (3 × 10 mL). Fmoc-Ala-OH (0.889 g, 2.857 mmol), HBTU (1.01 g, 2.655 mmol) and 1-hydroxybenzotriazol (HOBt) (0.393 g, 2.907 mmol) were dissolved in DMF (7 mL). DIPEA (0.818 g, 6.33 mmol) was added and the mixture was preactivated for 5 min and added to the resin. The mixture was then shaken for 2 h and afterward washed with DMF (4 × 10 mL), CH_2Cl_2 (2 × 10 mL), and MeOH (10 mL) and dried in vacuo. Residual aminopolystyrene groups were capped with 20% acetic anhydride for 1 h. The Fmoc-Ala-functionalized PS resin was treated with piperidine/DMF 1:4 (3 × 12 mL) for 2 × 2 min and 1 × 20 min and then washed with DMF (8 × 10 mL), CH_2Cl_2 (4 × 10 mL), and MeOH (2 × 10 mL) and dried in vacuo.

Compound **4** (0.165 g, 0.681 mmol), HBTU (0.262 g, 0.690 mmol) and HOBt (0.096 g, 0.708 mmol) were dissolved in DMF (5 mL), DIPEA (0.176 g, 1.360 mmol) was added and the mixture was preactivated for 5 min. The mixture was then added to the Ala-functionalized PS resin (0.347 mmol), shaken for 16 h, washed with DMF (4 × 10 mL), and then shaken with Ac_2O (0.387 g, 3.793 mmol) in DMF (4 mL) for 1 h. The resin was washed with DMF (8 × 10 mL), CH_2Cl_2 (4 × 10 mL), and MeOH (2 × 10 mL) and dried in vacuo.

Fmoc-Tyr-Gly-Gly-Phe-Leu-OH (10). To the T-BAL1-derivatized resin **9** (0.153 g, 0.055 mmol) were added H-Leu-O*t*Bu·HCl (0.132 g, 0.590 mmol), $NaBH_3CN$ (0.042 g, 0.675 mmol) and THF/MeOH/TMOF (4:1:5) (2 mL) and the mixture was shaken for 16 h, washed with DMF (8 × 10 mL), CH_2Cl_2 (4 × 10 mL), and MeOH (2 × 10 mL), and dried in vacuo. Fmoc-Phe-OH (0.438 g, 1.130 mmol) was dissolved in DMF/CH_2Cl_2 (1:9) (8 mL) and *N*,*N*′-diisopropylcarbodiimide (DIPCDI) (0.157 g, 1.240 mmol) was slowly added under continuous shaking. The slurry was then added to the derivatized resin (0.055 mmol), shaken for 16 h, washed with DMF (8 × 10 mL), CH_2Cl_2 (4 × 10 mL), and MeOH (2 × 10 mL), and dried in vacuo. The Fmoc-protected resin was treated with piperidine/DMF (1:4) (3 × 5 mL) for 2 × 2 min and 1 × 20 min, then washed with DMF (4 × 10 mL), CH_2Cl_2 (2 × 10 mL), and MeOH (1 × 10 mL), and dried in vacuo. Subsequent derivatization of the dipeptidyl resin was performed using standard SPPS protocol. Coupling reactions were carried out as follows: Fmoc-AA-OH (4 equiv) was dissolved in DMF and preactivated for 5 min with HBTU (3.8 equiv), HOBt (4 equiv), and DIPEA (7.8 equiv) and transferred to a filter syringe with the derivatized resin (1 equiv based on loading measurement). Before the final Fmoc deprotection, the loading of the peptidylresin was calculated to 0.1210 mmol/g according to the UV absorption from the dibenzofulvene−piperidine complex. The Leu-Enkephalin derivatized resin was subsequently subjected to various acidolytic conditions where the yields of released pentapeptide were measured by AAA. TOF ES MS$^+$ calcd ($C_{43}H_{47}N_5O_9$ (777.86)), found 778 (M^+).

Acidolytic Cleavage of Leu-Enkephalin from T-BAL1 (Table 1). Leu-Enkephalin-derivatized resin was divided into portions (5 × 20 mg), where acidolysis was carried out for 2 h using (A) 95%TFA (aq); (B) TFA/CH_2Cl_2 (1:1); (C) 5% TFA/CH_2Cl_2; (D) 1% TFA/CH_2Cl_2. The resins were washed with CH_2Cl_2 and MeOH and dried in vacuo. The content of residual peptide on the resins was determined by AAA, using the uncleaved fully derivatized peptidyl resin as reference.

Fmoc-Phe-Gly-Gly-SEt (12). To the T-BAL1-derivatized resin **9** (0.200 g, 0.074 mmol) were added H-Gly(SEt)$_3$·HCl (0.037 g, 0.139 mmol), $NaBH_3CN$ (0.023 g, 0.369 mmol) and THF/MeOH (4:1) (2 mL), and the mixture was shaken for 24 h, washed with DMF (8 × 10 mL), CH_2Cl_2 (4 × 10 mL), and MeOH (2 × 10 mL), and dried in vacuo. Fmoc-Gly-OH (0.442 g, 1.486 mmol) was dissolved in DMF/CH_2Cl_2 (1:9) (6 mL), and DIPCDI (0.092 g, 0.726 mmol) was slowly added under continuous shaking. The slurry was then added to the derivatized resin (0.019 mmol) and shaken for 20 h, washed with DMF (8 × 10 mL), CH_2Cl_2 (4 × 10 mL), and MeOH (2 × 10 mL), and dried in vacuo. The Fmoc-protected resin was treated with piperidine/DMF (1:4) (3 × 5 mL) for 2 × 2 min and 1 × 20 min, then washed with DMF (5 × 10 mL), CH_2Cl_2 (4 × 10 mL), and MeOH (1 × 10 mL) and dried in vacuo. Fmoc-Phe-OH (0.117 g, 0.302 mmol), HBTU (0.108 g, 0.285 mmol), and HOBt (0.041 g, 0.300 mmol) were dissolved in DMF (3 mL). DIPEA (0.082 g, 0.631 mmol) was added, and the mixture was preactivated for 5 min. The mixture was added to the derivatized resin (0.074 mmol), shaken for 1 h, washed with DMF (8 × 10 mL), CH_2Cl_2 (4 × 10 mL), and MeOH (2 × 10 mL), and dried in vacuo. To portions of the derivatized resin (3 × 20 mg) was added (A) TFA/H_2O (19:1), (B) TFA/CH_2Cl_2 (1:19), or (C) AcOH/CH_2Cl_2 (1:1) (1 mL), after 2 h it was washed with the cleavage mixture (2 × 1.5 mL) and CH_2Cl_2 (4 × 1.5 mL) and evaporated. HPLC t_R = 8.3 min (A, 100%; B, 75%; C, 81%), TOF ES-MS$^+$ calcd ($C_{30}H_{31}N_3O_5S$ (545.65)), found 546.21 (MH^+) and 568.19 ($M + Na^+$).

Fmoc-Phe-Gly-NHCH═C(SEt)$_2$ (13). To 20 mg of the derivatized resin **11** was added TFA/dry CH_2Cl_2 (1:99) (1 mL). After 2 h it was washed with the cleavage mixture (2 × 2 mL) and CH_2Cl_2 (3 × 1 mL) and evaporated. HPLC t_R = 8.3 min (8%) for the thioester and t_R = 9.3 min (92%) for the ketene dithio acetal protected peptide. TOF ES-MS$^+$ calcd ($C_{32}H_{35}N_3O_4S_2$ (589.77)), found 589.00 (M^+) and 612.20 ($M + Na^+$).

Fmoc-Lys(Boc)-Gly(OMe)$_2$ (14). To the T-BAL1-derivatized resin **9** (0.156 g, 0.056 mmol) in THF/MeOH/TMOF (4:1:5) containing 1% AcOH (2.5 mL) were added H-Gly(OMe)$_2$ (0.158 g, 1.50 mmol) and $NaCNBH_3$ (0.072 g, 1.15 mmol), and the mixture was shaken for 16 h, washed with DMF (8 × 10 mL), CH_2Cl_2 (4 × 10 mL), and MeOH (2 × 10 mL), and dried in vacuo. Fmoc-Lys(Boc)-OH (0.540 g, 1.15 mmol) was dissolved in DMF/CH_2Cl_2 (1:9) (10 mL), and DIPCDI (0.135 g, 1.07 mmol) was slowly added under continuous shaking. The slurry was then added to the acetal-derivatized resin (0.047 mmol), shaken for 18 h, and washed with DMF (8 × 10 mL), CH_2Cl_2 (4 × 10 mL), and MeOH (2 × 10 mL) followed by air-drying.

The derivatized resin was divided into portions (3 × 20 mg) to which was added (A) TFA/H_2O (19:1), (B) TFA/CH_2Cl_2 (1:1), or (C) TFA/CH_2Cl_2 (1:99) (1 mL). After 2 h the mixture was washed with the cleavage mixture (2 × 1.5 mL) and CH_2Cl_2 (2 × 2 mL). For B the washes were neutralized with 10% $NaHCO_3$ (aqueous) and evaporated. HPLC t_R = 6.8 min (A, 100%; B, 89%; C, 60%) for the unprotected peptide and t_R = 8.3 min (C, 40%) for the acetal- and Boc-protected peptide). ES-MS$^+$ calcd ($C_{30}H_{41}N_3O_7$ (555.66)), found 392.0 ($MH^+ - H_2O$ (imine)).

Optimized Reductive Amination of T-BAL3 Using Microwaves. H-Leu-O*t*Bu (20 equiv) or H-Gly-O*t*Bu (20 equiv) and $NaBH_3CN$ (20 equiv) were suspended in TMOF/MeOH (4:1) and added to the T-BAL3-derivatized resin. The mixture was irradiated with microwaves at 60 °C for 10 min. The solvents were removed by suction, and the resin washed with DMF (3 times), DCM (3 times), and DMF (3 times).

Acidolytic Cleavage of Leu-Enkephalin from T-BAL3. Initially, the protected peptidyl resin was Fmoc-deprotected with

piperidine.DMF (1:4). Small samples of Leu-Enkephalin-derivatized resin (approximately 10 mg) were treated with various acidic cleavage mixtures for 2 h at room temperature. The supernatant from each cleavage experiment was removed by suction, and the residual resin washed with CH_2Cl_2. The residual resins were analyzed by AAA.

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Supporting Information Available: Experimental general remarks. Experimental procedure for compound **3**. ^1H and ^{13}C NMR data on compound **5**. HPLC data (265 nm) on crude Fmoc-Tyr-Gly-Gly-Phe-Leu-OH, crude Fmoc-Gly-Leu-O*t*Bu, crude Fmoc-Phe-Gly-Gly-SEt, and crude Fmoc-Phe-Gly-NHCH═C$(SEt)_2$ released from T-BAL1 resin. Cleavage yields of H-Tyr-Gly-Gly-Phe-Leu-Gly-Gly-OH from T-BAL1 and T-BAL3 resins based on amino acid analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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