Preparation and Applications of Xanthenylamide (XAL) Handles for Solid-Phase Synthesis of *C*-Terminal Peptide Amides under Particularly Mild Conditions[1−3]

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[[9-[(9-Fluorenylmethyloxycarbonyl)amino]xanthen-2(or 3)-yl]oxy]alkanoic acid (XAL) handles have been prepared by efficient four-step routes from 2- or 3-hydroxyxanthone and coupled onto a range of amino-functionalized supports. The resultant XAL supports are the starting points for solid-phase peptide synthesis by Fmoc chemistry. Upon completion of chain assembly, *C*-terminal peptide amides are released in excellent yields and purities by use of low concentrations [1−5% (v/v)] of trifluoroacetic acid (TFA) in dichloromethane, often without a need for added carbocation scavengers. These cleavage conditions allow retention of all or a significant portion of *tert*-butyl type and related side-chain protecting groups, which subsequently may be removed fully in a solution process carried out at higher acid concentration. XAL supports are particularly useful for the synthesis of acid-sensitive peptides, including tryptophan-containing sequences that are known to be susceptible to yield- and/or purity-reducing alkylation side reactions. The effectiveness of this chemistry was shown with the syntheses of prothrombin (1−9), acyl carrier protein (65−74), *Tabanus atratus* adipokinetic hormone, fragments of the protein RHK 1, CCK-8 sulfate, and oxytocin. Furthermore, the application of XAL supports for the preparation of fully protected peptide amides has been demonstrated.

Work from our laboratories[6,7] and others[8] has provided several methods for the stepwise solid-phase synthesis of *C*-terminal peptide amides in conjunction with protection schemes using the base-labile N^{α}-9-fluorenylmethyloxycarbonyl (Fmoc) function.[9] The best approaches use appropriate *handles*,[10] which are in effect *N*-protected (aromatic) amino acids that can be coupled onto amino-functionalized supports and later serve as a starting point

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(1) Abbreviations: AA, amino acid residue (free or protected, depending on context); BOP, (benzotriazolyloxy)tris(dimethylamino)-phosphonium hexafluorophosphate; CCK-8, cholecystokinin octapeptide (residues 26−33); DME, 1,2-dimethoxyethane; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; DIEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; EtOAc, ethyl acetate; FABMS, fast atom bombardment mass spectrometry; Fmoc, 9-fluorenylmethyloxycarbonyl; Fmoc-NH_2, 9-fluorenylmethyl carbamate; HOAc, acetic acid; HOAt, 7-aza-1-hydroxybenzotriazole; HOBt, 1-hydroxybenzotriazole; HATU, *N*-[(dimethylamino)(1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HPLC, high-performance liquid chromatography; MBHA, *p*-methylbenzhydrylamine (resin); NMM, *N*-methylmorpholine; PAL, 5-[[(4-Fmoc-amino)methyl]-3,5-dimethoxyphenoxy]valeric acid handle; PEG-PS, polyethylene glycol−polystyrene (graft support); PyAOP, (7-azabenzotriazolyloxy)tris(pyrrolidino)phosphonium hexafluorophosphate; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; reagent A, TFA−CH_2Cl_2−dimethyl sulfide (14:5:1); reagent R, TFA−thioanisole−1,2-ethanedithiol−anisole (90:5:3:2); Tmob, 2,4,6-trimethoxybenzyl; TFA, trifluoroacetic acid; XAL, xanthenylamide, the title handles of this paper.

(2) Taken in part from the Ph.D. theses of Y. H., University of Minnesota, 1996, and M. C. M., University of Minnesota, 1994.

(3) Various portions of this work were reported in preliminary form at a variety of venues: (a) Barany, G.; Albericio, F. In *Peptides 1990: Proceedings of the Twenty-First European Peptide Symposium*; Giralt, E., Andreu, D., Eds.; ESCOM Science Publishers: Leiden, The Netherlands, 1991; pp 139−142. (b) Bontems, R. J.; Hegyes, P.; Bontems, S. L.; Albericio, F.; Barany, G. In *Peptides−Chemistry and Biology: Proceedings of the Twelfth American Peptide Symposium*; Smith, J. A., Rivier, J. E., Eds.; ESCOM: Leiden, The Netherlands 1992; pp 601−602. (c) Han, Y.; Munson, M. C.; Albericio, F.; Barany, G. In *Peptides−Biology and Chemistry: Proceedings of the 1992 Chinese Peptide Symposium*; Du, Y.-c., Tam, J. P., Zhang,Y.-s., Eds.; ESCOM Science Publishers: Leiden, The Netherlands, 1993; pp 301−304. (d) Han Y.; Barany, G. In *Innovation and Perspectives in Solid Phase Synthesis & Complementary Technologies: Biological & Biomedical Application;* Epton, R., Ed.; Mayflower Worldwide Ltd.: Birmingham, England, 1994; pp 525−526. (e) Kates, S. A.; Minor, C. A.; Albericio, F.; Han, Y.; Barany, G. In *Peptides 1994: Proceedings of the Twenty-Third European Peptide Symposium;* Maia, H., Ed.; ESCOM Science Publishers: Leiden, The Netherlands, 1995; pp 262−264.

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(6) (a) Albericio, F.; Barany, G. *Int. J. Pept. Protein Res.* **1987**, *30*, 206−216. (b) Albericio, F.; Kneib-Cordonier, N.; Biancalana, S.; Gera, L.; Masada, R. I.; Hudson, D.; Barany, G. *J. Org. Chem.* **1990**, *55*, 3730−3743. (c) Sharma, S. K.; Songster, M. F.; Colpitts, T. L.; Hegyes, P.; Barany, G.; Castellino, F. J. *J. Org. Chem.* **1993**, *58*, 4993−4996. (d) Albericio, F.; Barany, G. *Int. J. Pept. Protein Res.* **1993**, *41*, 307−312 and references cited in all of these papers.

(7) Hammer, R. P.; Albericio, F.; Gera, L.; Barany, G. *Int. J. Pept. Protein Res.* **1990**, *36*, 31−45 and references cited therein.

(8) Handles (or resin supports) directed toward peptide amides are reviewed thoroughly in ref 6b and include those developed by the following: (a) Sieber, P. *Tetrahedron Lett.* **1987**, *28*, 2107−2110. (b) Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787−3790. (c) Stüber, W.; Knolle, J.; Breiphol, G. *Int. J. Pept. Protein Res.* **1989**, *34*, 215−221. More recent developments were described by the following: (d) Patek, M.; Lebl, M. *Tetrahedron Lett.* **1991**, *32*, 3891−3894. (e) Echner, H.; Voelter W. In *Peptide Chemistry 1992: Proceedings of the 2nd Japan Symposium on Peptide Chemistry*; Yanaihara, N., Ed.; ESCOM Science Publishers: Leiden, The Netherlands, 1993; pp 113−115. (f) Chao, H.-G.; Bernatowicz, M. S.; Matsueda, G. R. *J. Org. Chem.* **1993**, *58*, 2640−2644. (g) Pinori, M.; Di Gregorio, G.; Mascagni, P. In *Innovation and Perspectives in Solid Phase Synthesis & Complementary Technologies: Biological & Biomedical Application*; Epton, R., Ed.; Mayflower Worldwide Ltd.: Birmingham, England, 1994; pp 635−638. (h) Noda, M.; Yamaguchi, M.; Ando, E.; Takeda, K.; Nokihara, K. *J. Org. Chem.* **1994**, *59*, 7968−7975. (i) Calmes, M.; Daunis, J.; David, D.; Jacquier, R. *Int. J. Pept. Protein Res.* **1994**, *44*, 58−60. (j) Ajayaghosh, A.; Pillai, V. N. R. *Tetrahedron Lett.* **1995**, *36*, 777−780. (k) Chan, W. C.; White, P. D.; Beythlen, J.; Steinauer, R. *J. Chem. Soc., Chem. Commun.* **1995**, 589−592. (l) Holmes, C. P.; Jones, D. G. *J. Org. Chem.* **1995**, *60*, 2318−2319.

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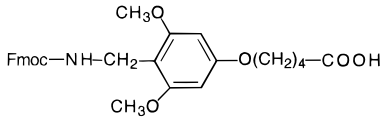
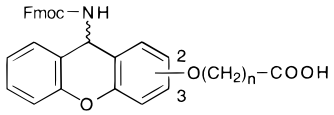
for chain elongation. Subsequently, cleavage of completed peptide chains containing the proper amide terminus is achieved by treatment with acid in the presence of suitable scavengers,[8a−f,h,i,k] or by photolysis in an appropriate solvent milieu.[7,8j,l,12] Particular success has been achieved with the tris(alkoxy)benzylamide (PAL) linkage,[6] which is established with the 5-[4-[(Fmoc-amino)methyl]-3,5-dimethoxyphenoxy]valeric acid handle (**1**) and is cleaved efficiently in the presence of trifluoroacetic acid (TFA) [~90% (v/v)].



1 PAL

The present paper reports on the preparation and applications of several [[(Fmoc-amino)xanthenyl]oxy]-alkanoic acids (**2a**,**b** and **3a**,**b**), which are handle variants (termed XAL) of the acid-labile xanthenyl protecting group for carboxamides.[13−15] In order to determine the optimal XAL handle, derivatives with different side-chain lengths (1 and 4 methylene groups, corresponding to acetic and valeric acids) and different substituent position (2 and 3, *meta* and *para*) to the xanthenyl system were prepared and evaluated. Cleavage of XAL handles to provide *C*-terminal peptide amides occurs at TFA concentrations [~1 to 25% (v/v)] which are lower than those required to effect cleavage of PAL. This property makes possible the direct preparation of protected peptide amides, as well as the synthesis of acid-sensitive peptide conjugates [illustrated herein for a sulfated peptide,[16] CCK-8]. Furthermore, a previously described tryptophan alkylation problem with PAL supports[6b] has been shown to be negligible for sensitive peptide amides synthesized on XAL supports. Finally, the compatibility of XAL chemistry with on-resin manipulation of cysteine residues has been delineated.



2a, n = 1 (2 position) **2-XAL$_1$**
2b, n = 4 (2 position) **2-XAL$_4$**
3a, n = 1 (3 position) **3-XAL$_1$**
3b, n = 4 (3 position) **3-XAL$_4$**

Results and Discussion

Preparation of XAL Handles. The required handles were prepared by efficient four-step routes (overall yield 50-66%), based in part on reasonable literature precedents and featuring direct transformations without separate purification steps for intermediates (Schemes 1 and 2). The respective starting materials, 2- or 3-hydroxyxanthone (**4**, **5**),[17] were obtained in two steps from *o*-anisoyl chloride plus 1,4- or 1,3-dimethoxybenzene, through the corresponding trimethoxybenzophenone intermediates (**6**, **7**). Alternatively, **4** was obtained by a new method in a single direct step from commercially available 2,2′-dihydroxy-4-methoxybenzophenone. The phenolic function of **4** or **5** was alkylated with ethyl bromoacetate (series **a**) or ethyl 5-bromovalerate (series **b**) to provide esters (**8**, **9**) which were saponified to the corresponding acids (**10**, **11**).[15,18] In the 2-XAL series, the carbonyls of **10a**,**b** were next reduced selectively with sodium borohydride in aqueous media at pH 8, to give xanthydrols **12a**,**b**. However, the same reactions in the 3-XAL series gave over-reduction to the corresponding (xanthen-3-yloxy)alkanoic acids **13a**,**b**, so the critical reduction step was carried out instead with Zn in the presence of NaOH, or with sodium amalgam. Regardless of how they were generated, xanthydrols were trapped under appropriate acidic conditions with 9-fluorenylmethyl carbamate (Fmoc-NH_2) (**14**)[8c] to give the title

(9) Reviews: (a) Barany, G.; Kneib-Cordonier, N.; Mullen, D. G. *Int. J. Pept. Protein Res.* **1987**, *30*, 705−739. (b) Atherton, E.; Sheppard, R. C. *Solid Phase Peptide Synthesis, A Practical Approach*; IRL Press at Oxford University Press: Oxford, U.K., 1989. (c) Fields, G. B.; Noble, R. L. *Int. J. Pept. Protein Res.* **1990**, *35*, 161−214. (d) Fields, G. B.; Tian, Z.; Barany, G. In *Synthetic Peptides. A User's Guide*; Grant, G. A., Ed.; W. H. Freeman and Co.: New York, 1992; pp 77−183.

(10) Handles are defined as bifunctional spacers which serve to attach the initial residue to the polymeric support in two discrete steps. One end of the handle incorporates features of a smoothly cleavable protecting group, and the other end allows facile coupling to a previously functionalized support. For reviews, see refs 9a, 9d, and the following: (a) Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Tetrahedron* **1993**, *49*, 11065−11133. (b) Barany, G.; Albericio, F. In *Peptides−Chemistry, Structure and Biology: Proceedings of the Thirteenth American Peptide Symposium*; Hodges, R. S., Smith, J. A., Eds.; ESCOM: Leiden, The Netherlands 1994; pp 1078−1080.

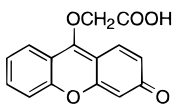
(11) For an excellent discussion of possible scavengers, see: King, D.; Fields, C.; Fields, G. *Int. J. Pept. Protein Res.* **1990**, *36*, 255−266. Further important information about scavengers appears in refs 6b and 28.

(12) Rich, D. H.; Gurwara, S. K. *Tetrahedron Lett.* **1975**, 301−304.

(13) (a) Shimonishi, Y.; Sakakibara, S.; Akabori, S. *Bull. Chem. Soc. Jpn.* **1962**, *35*, 1966−1970. (b) Dorman, L. C.; Nelson, D. A.; Chow, R. C. L. In *Progress in Peptide Research*; Lande, S., Ed.; Gordon and Breach: New York, 1972; Vol. 2, pp 65−68. (c) Solé, N. A.; Han, Y.; Vágner, J.; Gross, C. M.; Tejbrant, J.; Barany, G. In *Peptides−Chemistry, Structure and Biology: Proceedings of the Fourteenth American Peptide Symposium*; Kaumaya, P. T. P., Hodges, R. S., Eds.; Mayflower Scientific Ltd.: Kingswinford, England, 1996; pp 113−114.

(14) Sieber (ref 8a) has reported on a variant of XAL in which Merrifield chloromethyl-resin is alkylated by 3-hydroxyxanthone (**5**), followed by on-resin reduction by $LiBH_4$ in refluxing THF and acid-catalyzed trapping with Fmoc-NH_2 (**14**). While superficially similar to our chemistry for handle **3a** (Scheme 2), we had great difficulty reproducing the published on-resin procedures. Apparently, these difficulties have now been brought under control with a resin reported on by Chan *et al.* (ref 8k).

(15) Our initial studies (refs 3a,b) involved handles **2a** and **2b**, and we were unable to prepare **3a**. Subsequently, we became aware of the work of Caciagli, V.; Longobardi, M. G.; Pessi, A. Italian Patent Application, 19697A, March 16, 1990, SCLAVO SpA, Siena, Italy. These researchers introduced a Na/Hg reduction step to convert **11a** to **12a** [they noted, as do we, difficulties when borohydrides were used for reduction] and continued successfully to obtain **3a** which was then applied for the solid-phase synthesis of *C*-terminal amides. They also reported an unusual side reaction during the alkylation of **5** *directly* with chloroacetic acid; besides the desired **11a**, the isomer shown below was formed from reaction of the 9-OH, 3-oxo tautomer of **5**.

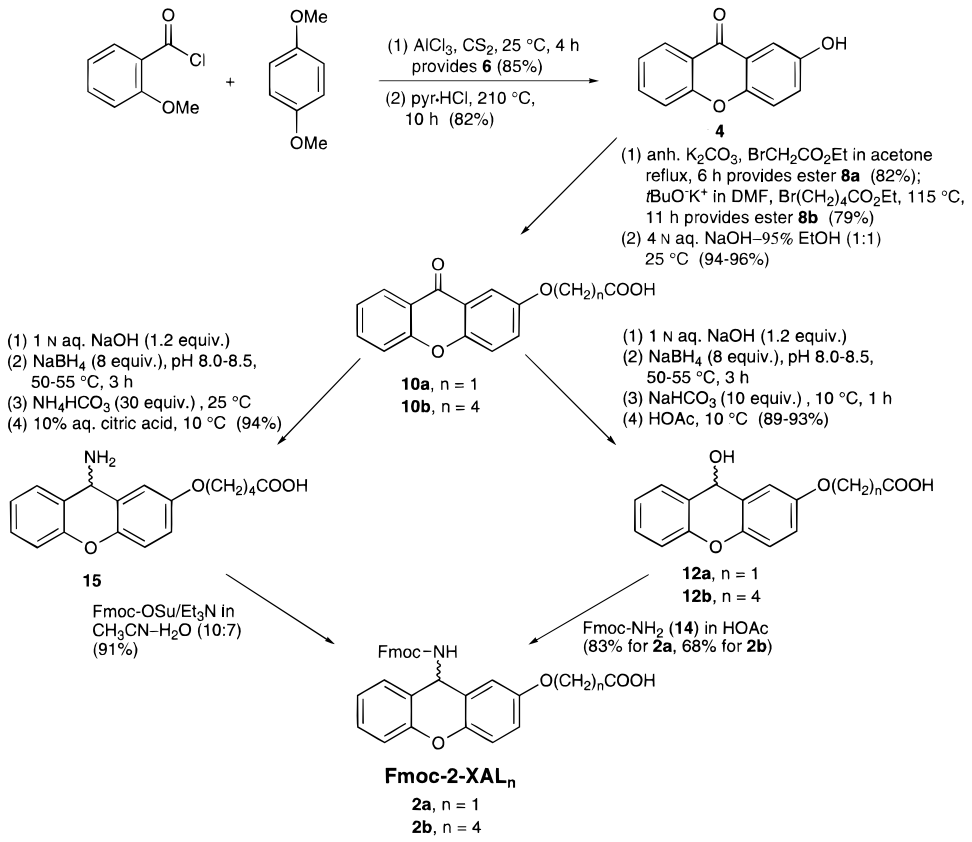


(16) As reviewed in ref 9d, **s**ynthesis of Tyr sulfate-containing peptides is difficult, due to the substantial acid lability of the sulfate ester.

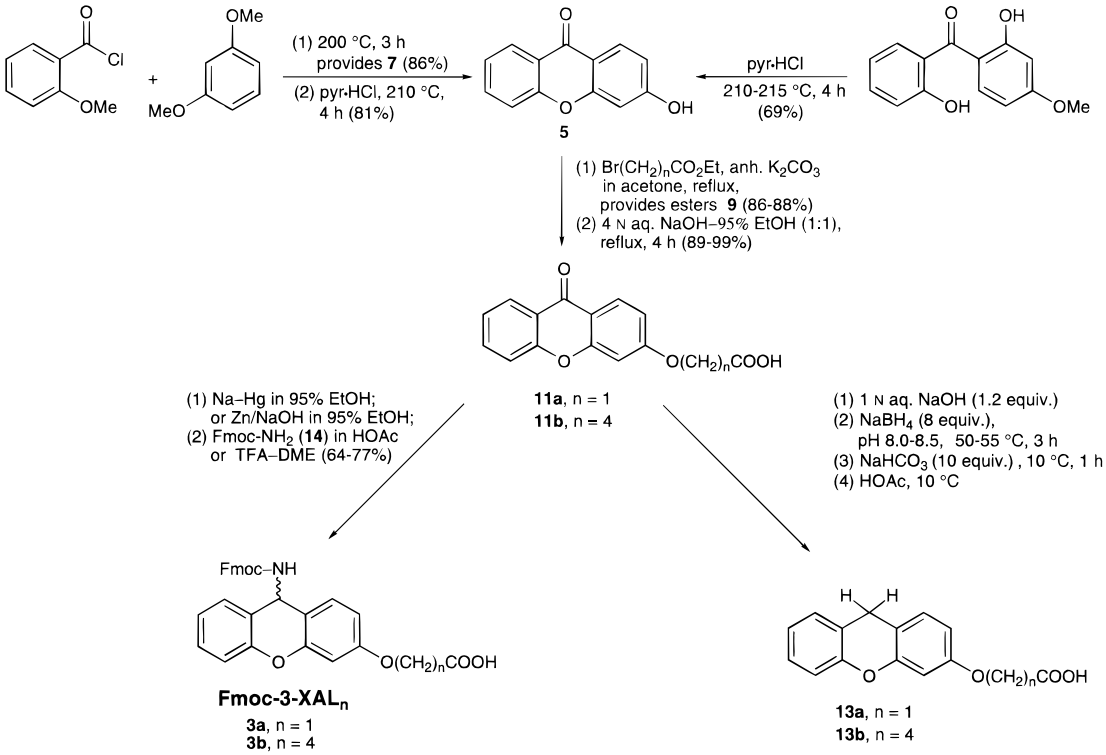
(17) Routes to the various isomeric hydroxyxanthones were first reported in the late 19th/early 20th century German literature, and some of these compounds have also been isolated as natural products. For a summary, see: (a) *Dictionary of Organic Compounds*, H-03557 through H-03560; Chapman & Hall: New York, 1982; Vol. 3, 5th ed.; pp 3265−3266. More recent, relevant articles include the following: (b) Van Allan, J. A. *J. Org. Chem.* **1958**, *23*, 1679−1682. (c) Royer, R.; Lechartier, J. P.; Demerseman, P. *Bull. Soc. Chim. Fr.* **1971**, 1707−1710. For the present work, the procedure developed in ref 17b for 3-hydroxyxanthone (**5**) was modified and further optimized to prepare both **5** and 2-hydroxyxanthone (**4**) in more convenient ways (compare to ref 17c, which has sketchy experimental details).

(18) Some of these esters and acids are known, see ref 15 and (a) Cassella Farbwerke Mainkur A.-G. Brit. Pat. 923,132, Apr 10, 1963; Ger. Appl., Jan 20, 1960 [*Chem. Abstr.* **1963**, *59*, p9994b]. (b) Puranik, G. S.; Agasimundin, Y. S.; Rajagopal, S. *Indian J. Appl. Chem.* **1968**, *31*, 105−107. (c) Somlai, C.; Nyerges, L.; Penke, B.; Hegyes, P.; Voelter, W. *J. Prakt. Chem./Chem.-Ztg.* **1994**, *336*, 429−433.

Scheme 1



Scheme 2



compounds **2a**,**b** and **3a**,**b**. An alternative route, most practical in the 2-XAL series,[19] transformed **10b** to the corresponding amino derivative **15**, which was converted to the final handle **2b** by reaction with *N*-(Fmoc-oxy)-succinimide (Fmoc-OSu).[20]

Use of XAL Handles in Solid-Phase Peptide Synthesis. Handle derivatives **2a**,**b** and **3a**,**b** were introduced readily onto a range of amino-functionalized supports. Quantitative anchoring was mediated conveniently by the phosphonium and uronium salt derivatives[21] of 1-hydroxybenzotriazole (HOBt) or 7-aza-1-hydroxybenzotriazole (HOAt), in the presence of *N*-methylmorpholine (NMM) or *N*,*N*-diisopropylethylamine (DIEA), with *N*,*N*-dimethylformamide (DMF) as

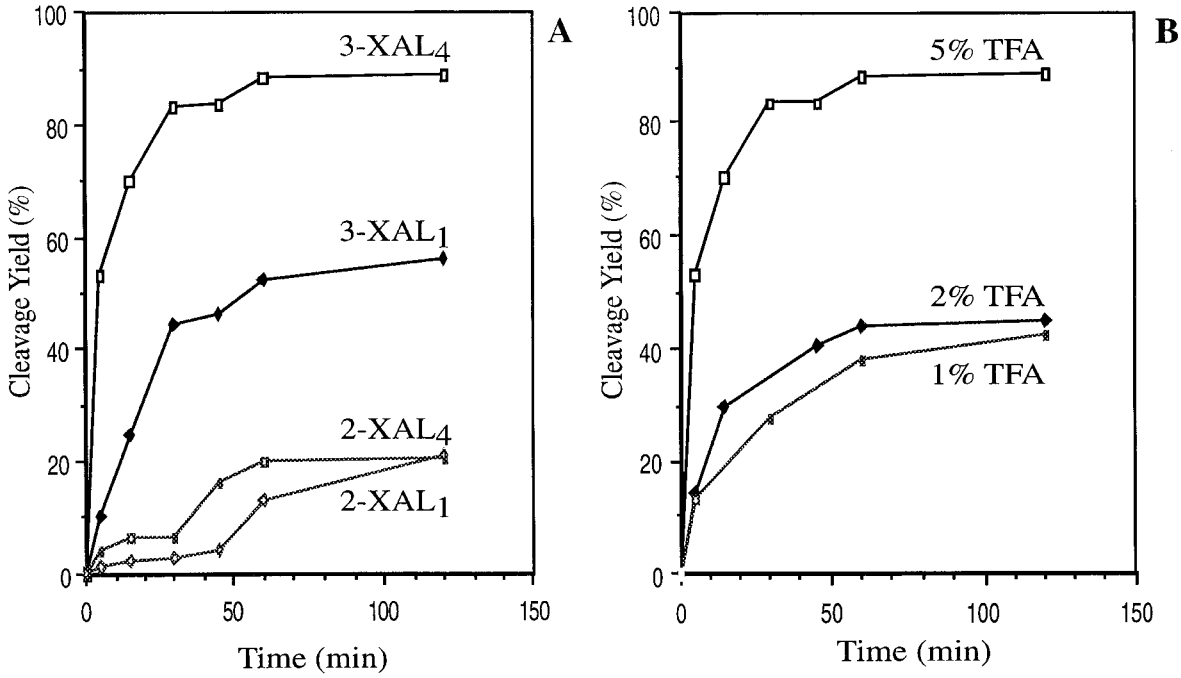


Figure 1. (A) Kinetics of cleavage for four H-Leu-Ala-Gly-Val-XAL-Phe-PEG-PS supports by TFA−CH_2Cl_2 (1:19) at 25 °C. (B) Kinetics of cleavage of H-Leu-Ala-Gly-Val-3-XAL_4-Phe-PEG-PS as a function of TFA concentration.

solvent. Parent supports used included standard polystyrene (PS) resins, as well as polyethylene glycol−polystyrene (PEG-PS) graft supports.[22] The Fmoc group was removed from XAL handle resins, and stepwise elaboration of peptide chains by Fmoc chemistry proceeded normally according to standard manual or automated protocols.[6b,9d,23]

The labilities of various XAL anchors to *acid* were assessed by model kinetic studies after assemblies of the Merrifield tetrapeptide (Leu-Ala-Gly-Val).[24] Cleavage by TFA−CH_2Cl_2 (1:19) was most efficient for 3-XAL_4 (**3b**: 87% cleavage in 1 h). The 3-XAL_1 (**3a**) anchor with a shorter spacer ($n = 1$) was cleaved 2-fold more slowly, and cleavage of 2-XAL (**2a**,**b**) derivatives was slower by an order of magnitude (Figure 1A). The most labile linkage, derived from 3-XAL_4 (**3b**), was cleaved even with a very low concentration of TFA (1%); moderate yields were observed (~40%; see Figure 1B).

The kinetic studies suggest three alternative ways in which XAL-anchored peptides can be released, depending on the synthetic objective. To obtain protected peptide amides, low concentrations of TFA are used (see next section), and when free unprotected peptide amides are required, the cleavage/deprotection is carried out either in a two-stage process ("low/high" acid) or in a single step at higher TFA concentration ("high" acid; illustrations follow).

Preparation of Protected Peptide Amides with 3-XAL. When TFA−CH_2Cl_2 (1:99) is used as the cleavage cocktail, *tert*-butyl-type protecting groups that are typical for Fmoc SPPS are not removed. However, the aforementioned dilute acid reagent is not strong enough to promote full cleavage of the most labile XAL anchor, i.e., that derived from 3-XAL_4 (**3b**), either (see Figure 1B). The unsatisfactory saturation of cleavage yield is apparently due to buffering of TFA by amide bonds and other functional groups in peptides.[25] To overcome this problem, we employ repeated short pulses with TFA−CH_2Cl_2 (1:99). The protected peptide amide Fmoc-Ala-Pro-Trp-Ala-Val-Leu-Glu(O^{t}Bu)-Val-Ala-NH_2 was obtained in 84% yield and excellent purity (>90%) after six consecutive 5 min treatments with TFA−CH_2Cl_2 (1:99) (Figure 2).

Preparation of Unprotected Peptide Amides with XAL. In a two-stage process, initial treatment in the presence of 1−25% TFA released peptides in high yields,

(19) This alternative route for the 3-XAL series led to the desired amine derivative, but it remained contaminated with the xanthydrol intermediate. Acylation of the amine component in these product mixtures with Fmoc-OSu provided final XAL handles (**3a**,**b**) which were difficult to obtain absolutely free of xanthydrol or its decomposition products.

(20) Ten Kortenaar, P. B. W.; van Dijk, B. G.; Peters, J. M.; Raaben, B. J.; Adams, P. J. H. M.; Tesser, G. I. *Int. J. Pept. Protein Res.* **1986**, *27*, 398−400.

(21) The development and applications of the relevant coupling reagents are reported by the following: (a) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219−1222. (b) Dourtoglou, V.; Ziegler, J.-C.; Gross, B. *Tetrahedron Lett.* **1978**, 1269−1272. (c) Hudson, D. *J. Org. Chem.* **1988**, *53*, 617−624. (d) Fournier, A.; Wang, C. T.; Felix, A. M. *Int. J. Pept. Protein Res.* **1988**, *31*, 86−97. (e) Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927−1930. (f) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398. (g) Carpino, L. A.; El-Fahan, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201−203. (h) Kates, S. A.; Minor, C. A.; Shroff, H.; Haaseth, R. C.; Triolo, S.; El-Faham, A.; Carpino, L. A.; Albericio, F. In *Peptides 1994: Proceedings of the Twenty-Third European Peptide Symposium*; Maia, H. L. S., Ed.; ESCOM: Science Publishers: Leiden, The Netherlands, 1995; pp 248−249.

(22) (a) Barany, G.; Albericio, F.; Solé, N. A.; Griffin,, G. W.; Kates, S. A.; Hudson, D. In *Peptides 1992: Proceedings of the Twenty-Second European Peptide Symposium*; Schneider C. H., Eberle, A. N., Eds.; ESCOM Science Publishers: Leiden, The Netherlands, 1993; pp 267−268. (b) Zalipsky, S.; Chang, J. L.; Albericio, F.; Barany, G. *React. Polym.* **1994**, *22*, 243−258 and references cited therein.

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(24) (a) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149−2154. (b) Kent, S. B. H.; Mitchell, A. R.; Barany, G.; Merrifield, R. B. *Anal. Chem.* **1978**, *50*, 155−159.

(25) This "buffering" phenomenon was first discovered and addressed by Riniker, B.; Flörsheimer, A.; Fretz, H.; Sieber, P.; Kamber, B. *Tetrahedron* **1993**, *49*, 9307−9320.

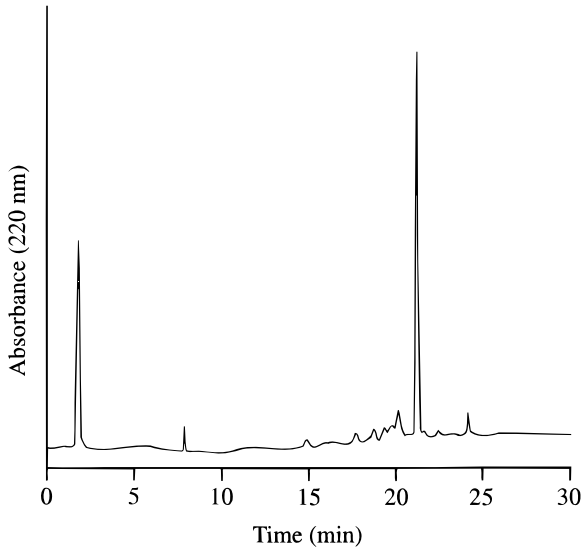

Figure 2. Analytical HPLC of *crude* Fmoc-Ala-Pro-Trp-Ala-Val-Leu-Glu(O^{t}Bu)-Val-Ala-NH_2 prepared starting with Fmoc-3-XAL_4-PEG-PS (derived from handle **3b**; further details in the Experimental Section). HPLC was performed on a Delta Pak C_{18} reversed-phase column (3.9 × 150 mm, 5 μm), with a linear gradient using 0.036% TFA in CH_3CN and 0.045% aqueous TFA, from 1:9 to 1:0 over 20 min, flow rate 1.0 mL/min.

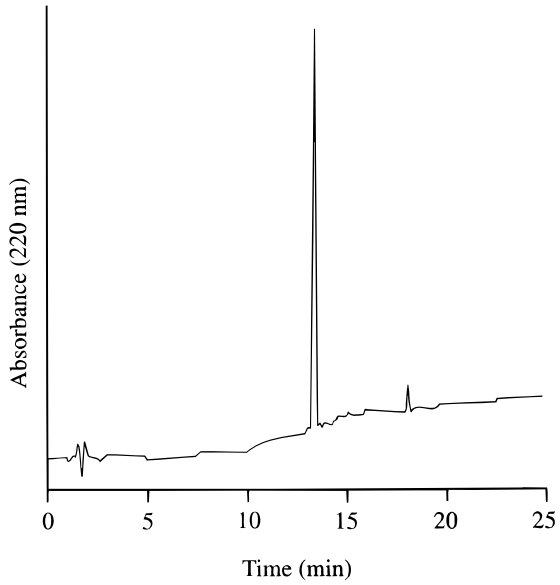

Figure 3. Analytical HPLC of *crude* prothrombin fragment (1−9), H-Ala-Asn-Lys-Gly-Phe-Leu-Glu-Glu-Val-NH_2 prepared on Fmoc-3-XAL_4-PEG-PS support (0.21 mmol/g, derived from handle **3b**), after peptide chain assembly and cleavage with reagent R for 2 h at 25 °C. Couplings of appropriately protected Fmoc-amino acid derivatives were performed automatically on a continuous-flow PerSeptive Biosystems 9050 peptide synthesizer, using an HATU/DIEA protocol (Table 2). HPLC was performed as for Figure 2, except the gradient was over 30 min.

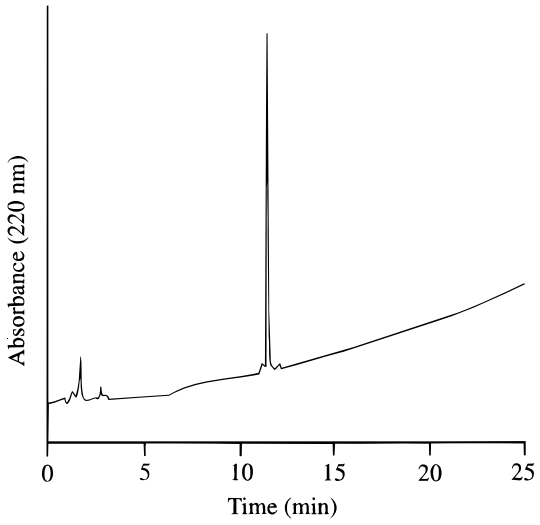

Figure 4. Analytical HPLC of *crude* acyl carrier protein (65−74), H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-NH_2 prepared on Fmoc-3-XAL_4-PEG-PS support (derived from handle **3b**). All other details identical to those of Figure 3. The slight shoulder just before the main peak is the des(Val) species, whereas the des(Asn) species elutes just after the main peak.

but with partial retention of side-chain *tert*-butyl protection (e.g., see Figure 5A later). Therefore, a second step followed in which the acid (TFA) concentration was increased to an overall level of 50%, so that full deprotection was achieved in solution.[26] Similar overall yields and purities of free *C*-terminal peptide amides were also observed when higher acid concentrations, as usually applied at the end of standard Fmoc syntheses, were used for direct cleavage/deprotection in a single step. This approach was demonstrated by syntheses of fragment 1−9 of prothrombin [Figure 3: anchor derived from 3-XAL_4 (**3b**), 97% cleavage with reagent R, TFA−thioanisole−1,2-ethanedithiol−anisole (90:5:3:2), 2 h, 25 °C] and the (65−74) fragment[27] of the acyl carrier protein [Figure 4: anchor derived from 3-XAL_4 (**3b**), 95% cleavage with reagent R].

Preparation of Tryptophan-containing Peptides with XAL.[28] The sequence pGlu-Leu-Thr-Phe-Thr-Pro-Gly-Trp-NH_2, derived from *Tabanus atratus* adipokinetic hormone,[29] was developed previously as a sensitive and quantifiable model for a tryptophan alkylation side reaction with PAL supports.[6b] Cleavage of peptide-PAL-Nle-MBHA-PS with reagent R for 2 + 8 h proceeded in only ∼55% yield, with the uncleaved peptide presumed to be linked to resin-bound PAL *via* the 2-position of the indole side chain. Alternatively, cleavage of peptide-PAL-MBHA-PS (omitting the intervening Nle residue which provides an acid-stable point of linkage) with reagent R or a variety of other acid reagent/scavenger cocktails resulted in not only the desired peptide but also substantial amounts (nearly equimolar, and often higher) of the tryptophan-alkylated byproduct being released into solution. For these experiments, the byproduct, which included a PAL-NH_2 moiety, was characterized by NMR and FABMS. In contrast to the just-mentioned results with PAL, the present paper shows that cleavage of peptide-2-XAL_4-Nle-MBHA-PS (derived from handle **2b**)

(26) To obtain Arg-containing free peptides that were originally protected with either Pmc or Pbf, it is necessary to remove CH_2Cl_2 and add neat TFA in the presence of scavengers.

(27) The acyl carrier protein (65−74) decapeptide is considered a "difficult" sequence for stepwise chain assembly and was described first by Hancock, W. S.; Prescott, D. J.; Vagelos, P. R.; Marshall, G. R. *J. Org. Chem.* **1973**, *38*, 774−781.

(28) For a systematic study of cleavage conditions and a comparison of the relative utility of 2-XAL_4 *vs* PAL supports for the preparation of indolicidin [a 13-residue peptide amide with five Trp residues], see: Van Abel, R. J.; Tang, Y.-Q.; Rao, V. S. V.; Dobb, C. H.; Tran, D.; Barany, G.; Selsted, M. E. *Int. J. Pept. Protein Res.* **1995**, *45*, 401−409.

(29) Jaffe, H.; Raina, A. K.; Riley, C. T.; Fraser, B. A.; Nachman, R. J.; Vogel, V. W.; Zhang, Y. S.; Hayes, T. K. *Proc. Nat. Acad. Sci. U.S.A.* **1989**, *86*, 8161−8164.

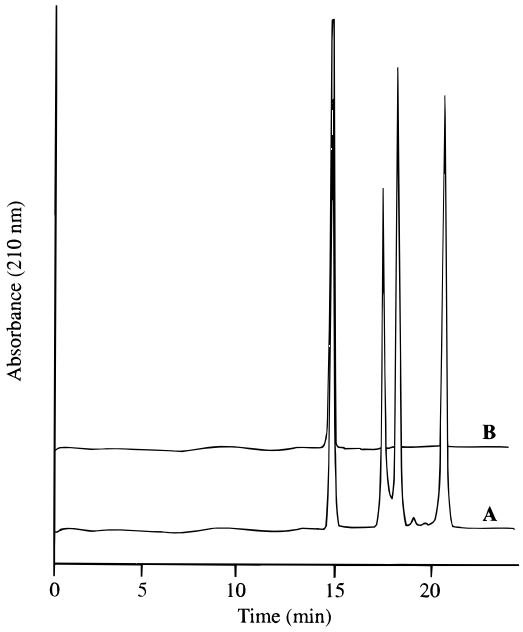

Figure 5. Analytical HPLC of *crude* samples of *T. atratus* adipokinetic hormone (ref 29) prepared on Fmoc-2-XAL$_4$-PEG-PS support (derived from handle **2b**; further details in the Experimental Section). HPLC was performed on a Vydac C_{18} reversed-phase column (4.6 × 250 mm, 5 μm), with a linear gradient over 20 min, using CH_3CN and 0.01 N HCl from 1:9 to 3:2; flow rate 1.2 mL/min. Panel A: sample obtained after initial cleavage with TFA−CH_2Cl_2 (1:19) for 2 h at 25 °C. Panel B: sample obtained after adding neat TFA to bring the overall filtrate to TFA−CH_2Cl_2 (1:1) for 2 h at 25 °C.

with reagent R provides the desired peptide in excellent purity and yield (>90%), meaning that any putative tryptophan alkylation side reaction would occur at a relatively low level. Even more striking, the purities and yields upon cleavage of XAL did not seem to require the scavengers, individual or in combination, as had been optimized in the PAL series.[6b] Thus, release from the support was achieved adequately with TFA−CH_2Cl_2 (1:19) for 1 h at 25 °C, and TFA−CH_2Cl_2 (1:1) for 1 h further in solution was needed to deblock fully the identifiable two mono-*tert*-butyl and single di-*tert*-butyl intermediates (Figure 5).

A further demonstration of negligible back-alkylation in the XAL series was obtained when two fragments of the protein RHK 1[30] [H-Ser-Ser-Ile-Pro-Asp-Ala-Phe-Trp-NH_2 (458−465) and H-Ser-Ser-Ile-Pro-Asp-Ala-Phe-Trp-Trp-NH_2 (458−466)] containing one and two residues of Trp, respectively, at the *C*-terminal end, were synthesized in parallel on PAL *vs* 3-XAL$_4$ (derived from handle **3b**) derivatized PEG-PS supports. Cleavage of peptide-resins in the absence of scavengers [TFA−CH_2Cl_2 (9:1)] gave in both cases better yields when XAL resins were used (Table 1, entries 1 and 2). Furthermore, the presence of scavengers (Table 1, entries 3, 4, 6, 7) did not improve significantly the cleavage yield, nor did lower amounts of TFA (Table 1, entry 5) decrease the yields. HPLC analyses of the crude cleaved peptides showed better homogeneities when starting with XAL handles (Figure 6).

Preparation of CCK-8 Sulfate with XAL. The title sulfated octapeptide[31] is recognized to be a challenging synthetic target due to the presence of several acid- and/or alkylation-sensitive residues (Trp, Met), as well as the acid-labile sulfate on tyrosine. In the present work, Fmoc-3-XAL$_1$-PEG-PS (derived from handle **3a**) was used as the starting point to assemble the linear sequence by standard Fmoc chemistry. Incorporation of the tyrosine sulfate residue, as its N^{α}-Fmoc, side-chain barium salt, was accomplished directly by a BOP/HOBt/NMM coupling. The two aspartate residues were protected as allyl esters and deprotected upon completion of chain assembly by use of Pd(PPh_3)$_4$ in $CHCl_3$−HOAc−NMM (20:1:0.5), for 2 h at 25 °C.[32] The overall cleavage yield, using TFA−CH_2Cl_2−H_2O (1:18:1) for 15 min at 25 °C, was 71%, and the desired product represented >95% of the total peptide material (Figure 7A). This strategy represented a clear improvement over our previous result[3b] starting with Fmoc-2-XAL$_4$-PEG-PS (derived from handle **2b**) and using ^{t}Bu ester protection for the two aspartate residues. A number of cleavage conditions were examined, and TFA−CH_2Cl_2−β-mercaptoethanol−anisole (50:45:3:2), 15 min, 25 °C, was found to represent the optimal compromise between acid-promoted loss of sulfate and incomplete deprotection. The overall cleavage yield was 90%, and the desired product represented ∼70% of the total peptide material (Figure 7B). Also observed from this earlier work were 10% of unsulfated CCK-8 and 20% of CCK-8 sulfate derivatives with one or the other of the aspartates retaining *tert*-butyl side-chain protection.

Table 1. Acidolytic Cleavage of PAL and XAL Anchoring Linkages for Peptides with *C*-Terminal Tryptophan[a]

entry	peptide[b,c]	cleavage cocktail[d]	cleavage yield,[e] % PAL	XAL
1	H-SSIPDAFW-NH_2	TFA−CH_2Cl_2 (9:1)	14	89
2	H-SSIPDAFWW-NH_2	TFA−CH_2Cl_2 (9:1)	33	92
3	H-SSIPDAFW-NH_2	reagent R[d]		96
4	H-SSIPDAFW-NH_2	TFA−H_2O (9:1)		95
5	H-SSIPDAFW-NH_2	TFA−CH_2Cl_2 (2:3)		91
6	H-SSIPDAFWW-NH_2	reagent R[d]		96
7	H-SSIPDAFWW-NH_2	TFA−H_2O (9:1)		94

[a] The rationale of these studies is explained in the text. [b] Peptides were assembled on Fmoc-3-XAL$_4$-PEG-PS (0.21 mmol/g; derived from handle **3b**). Couplings of appropriately protected Fmoc-amino acid derivatives were performed automatically on a continuous-flow PerSeptive Biosystems 9050 peptide synthesizer, using an HATU/DIEA protocol (Table 2). All cleavages were carried out for 1 h at 25 °C, using the cleavage cocktail in column 3. [c] Representative analytical HPLC data shown in Figure 6. [d] All cleavage cocktails were prepared freshly immediately before use. Reagent R is TFA−thioanisole−1,2-ethanedithiol−anisole (90:5:3:2). [e] Cleavage yields were calculated on the basis of amino acid analyses of peptide-resins before and after the cleavage, with respect to norleucine serving as "internal reference" amino acid.

Preparation of Oxytocin with XAL. The target sequence was of interest as a test of the integration of the XAL approach with our experiences on thiol protection and disulfide bond formation.[33,34] The linear se-

(30) Tamkun, M.; Knoth, E. M.; Walbridge, J. A.; Kroemer, H.; Roden, D. M.; Glover, D. M. *FASEB J.* **1991**, *5*, 331−337.

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(33) (a) García-Echeverría, C.; Albericio, F.; Pons, M.; Barany, G.; Giralt, E. *Tetrahedron Lett.* **1989**, *30*, 2441−2444. (b) Albericio, F.; Hammer, R. P.; García-Echeverría, C.; Molins, M. A.; Chang, J. L.; Munson, M. C.; Pons, M.; Giralt, E.; Barany, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 402−413. (c) Review: Andreu, D.; Albericio, F.; Solé, N. A.; Munson, M. C.; Ferrer, M.; Barany, G. In *Methods in Molecular Biology, Vol. 35*: *Peptide Synthesis Protocols*; Pennington, M. W., Dunn, B. M., Eds.; Humana Press: Totowa, NJ, 1994; pp 91−169.

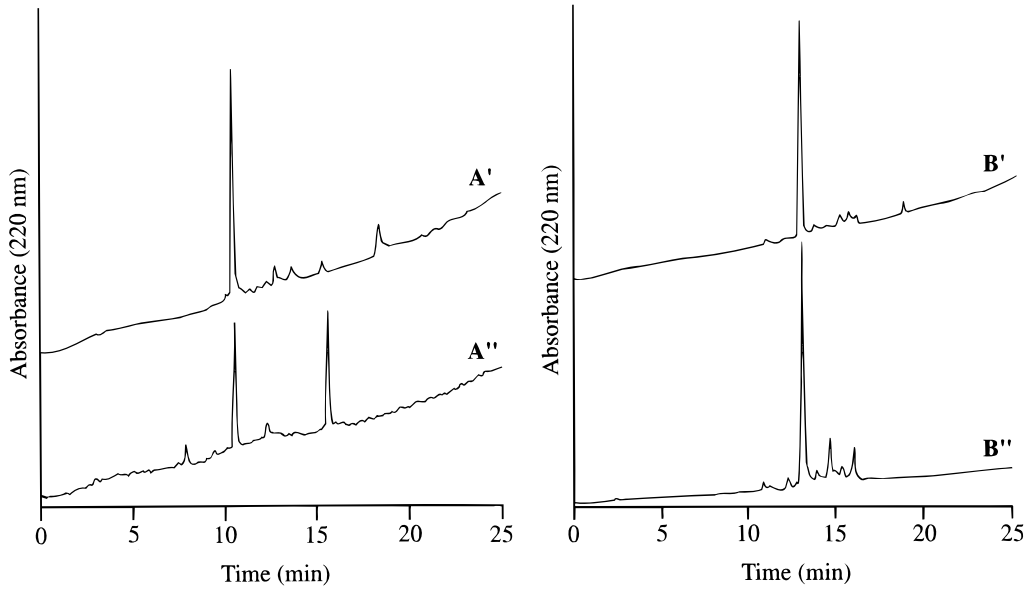

Figure 6. Analytical HPLC of *crude* samples of fragments (458−465) [panel A] and (485−466) [panel B] of protein RHK 1 (H-Ser-Ser-Ile-Pro-Asp-Ala-Phe-Trp-NH_2 and H-Ser-Ser-Ile-Pro-Asp-Ala-Phe-Trp-Trp-NH_2, respectively). Details of the syntheses are in the footnotes to Table 1, and HPLC was performed as for Figure 3. In each case, syntheses marked with a prime started with Fmoc-3-XAL_4-PEG-PS derived from handle **3b**, and syntheses marked with a double prime started with Fmoc-PAL-PEG-PS.

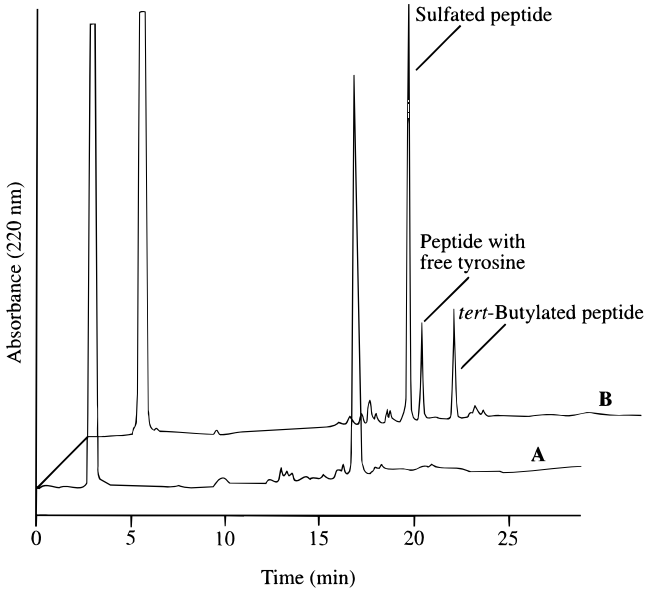

Figure 7. Analytical HPLC of *crude* samples of CCK-8 sulfated peptide (details of syntheses are in the Experimental Section). Panel A: starting with Fmoc-3-XAL_1-PEG-PS (derived from handle **3a**). HPLC was performed similar to that described in the legend to Figure 2, except the gradient over 20 min ranged from 1:9 to 2:3. Panel B: starting with Fmoc-2-XAL_4-PEG-PS (derived from handle **2b**). HPLC was performed similar to that described in the legend to Figure 5, except the gradient over 20 min ranged from 1:9 to 2:3 and the flow rate was 1.0 mL/min.

quence was assembled smoothly on an Fmoc-2-XAL_4-Ala-MBHA support derived from handle **2b** (Scheme 3). Our *S*-2,4,6-trimethoxybenzyl (Tmob)[34a] protecting group was used to block the β-thiol group of cysteine, while ^{t}Bu was used for the phenol of tyrosine, and side chains of asparagine and glutamine remained unprotected. In some experiments, final cleavage was achieved with low concentrations of TFA, i.e., TFA−CH_2Cl_2−Et_3SiH (7:92:1) for 1 h at 25 °C (95% cleavage yield). Surprisingly, the expected dihydrooxytocin product was accompanied by a second, minor (∼25%) peak on HPLC (Figure 8A). When a higher concentration of TFA was used, i.e., TFA−CH_2Cl_2−Et_3SiH (70:29:1), the ratio of the two peaks was inverted (∼5:2) (Figure 8B). The additional peak was identified by FABMS and dithiothreitol reduction studies (Figure 8C) as cyclized oxytocin. Thus, high concentrations of TFA seem to favor direct formation of the disulfide upon XAL cleavage; as the concentration of acid is decreased, reduced product is favored. Shorter cleavage times, e.g., 30 min, resulted in almost exclusive formation of reduced product even with high levels of TFA. Such phenomena have *not* been observed previously with PAL and are not understood at present.

We also tested oxidative cleavage/cyclization of Cys(Tmob) while the protected peptide was still anchored on the resin. Reagents used were thallium(III) trifluoroacetate [1.1−1.3 equiv in DMF−anisole (19:1)], as well as iodine [3 equiv in TFA−CH_2Cl_2−anisole (82:15:3)].[33] In most cases, the absolute yields (∼40%) were comparable to those obtained with PAL-anchored peptides.[33b,34] HPLC traces were remarkably clean (upwards of 90% pure for crude cleavage, see Figure 9), and cleavage yields were almost always 90% or higher. Due to the direct formation of oxytocin as the cyclized disulfide upon cleavage of XAL anchors at higher TFA concentrations (see previous paragraph), it is difficult to know to what extent these good results reflect effective on-resin oxidation.

Conclusions

This paper has described optimal chemistries for preparation of several XAL handles. The crucial step is reduction of a xanthone carbonyl, a transformation which is prone to side reactions and difficult to achieve quantitatively and reproducibly in the solid-phase mode.[14,15] Consequently, our preformed handle approach,[6,7,10] in

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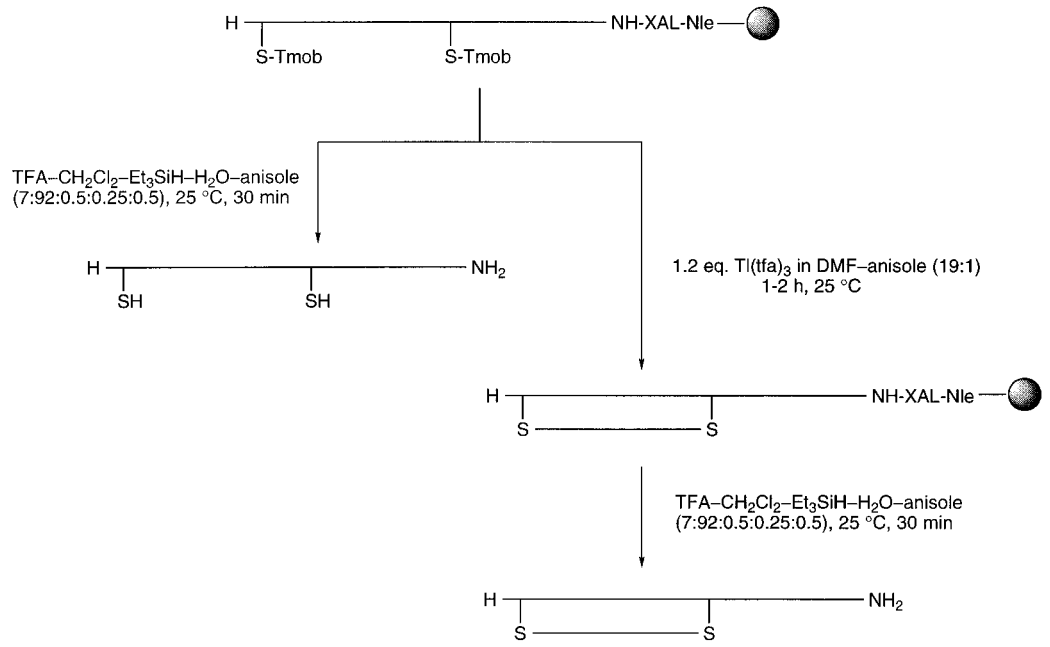
Scheme 3



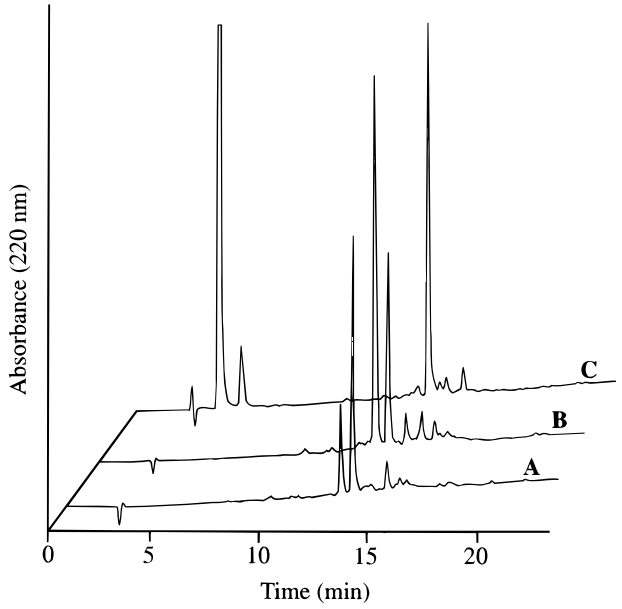


Figure 8. Analytical HPLC of *crude* samples of oxytocin prepared starting with Fmoc-2-XAL$_4$-PEG-PS (derived from handle **2b**; further details in the Experimental Section). HPLC was performed similar to that described in the legend to Figure 5, except the gradient over 20 min ranged from 1:9 to 2:3. Panel A: sample obtained when a low concentration of TFA was used [TFA−CH_2Cl_2−Et_3SiH (7:92:1) for 1 h at 25 °C]. Panel B: sample obtained when a higher concentration of TFA was used [TFA−CH_2Cl_2−Et_3SiH (70:29:1) for 1 h at 25 °C]. Panel C: material from sample B was concentrated and redissolved in 0.01 M $NaHCO_3$, pH ∼9, and solid dithiothreitol (∼5−8 equiv) was added; reduction was carried out for 1 h at 40 °C followed by chromatography.

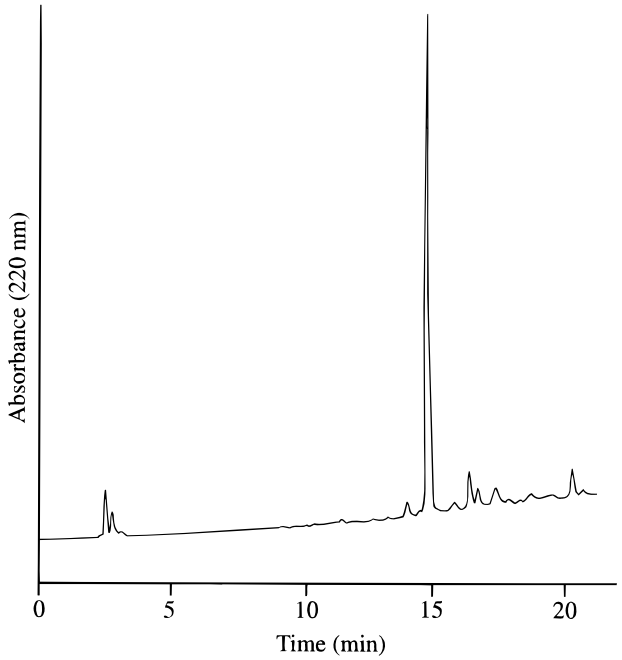


Figure 9. Analytical HPLC of *crude* cyclized oxytocin prepared starting with Fmoc-2-XAL$_4$-PEG-PS (derived from handle **2b**; further details in the Experimental Section). HPLC was exactly as in Figure 8.

which synthesis, purification, and characterization in solution precede smooth incorporations onto the support, is particularly appropriate in the XAL system. Additionally, the availability of handles allows the anchoring chemistry to be adapted to a wide range of useful solid-phase synthesis supports.[9,22]

The 3-XAL$_4$ (**3b**) handle containing a four-carbon spacer in position 3 of the xanthenyl ring appears to be optimal for the preparation of both free and protected *C*-terminal peptide amides. Final cleavage from the support can be accomplished with dilute TFA (1−5%) in CH_2Cl_2. In contradistinction to experiences with other handles, scavengers such as dimethyl sulfide, 1,2-ethanedithiol, anisole, and/or thioanisole are not required for high cleavage yields, nor for the optimal purity of Trp-containing peptides [note that scavengers are still needed for removal Pmc,[35] Pbf,[36] and/or Tmob[34] from peptides].

(35) Ramage, R.; Green, J.; Blake, A. J. *Tetrahedron* **1991**, *47*, 6353−6370.

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Experimental Section

Most of the materials and general synthetic and analytical procedures have been described in our earlier publications.[6,7,10,22,23,32] ^{1}H NMR spectra were recorded at 300 MHz on either a Nicolet NT-300 WB or an IBM NR/300 instrument and at 200 MHz on an IBM NR/200 instrument, using either $CDCl_3$ or CD_3SOCD_3 as solvent. ^{13}C NMR spectra were obtained at 75 or 50 MHz on the same instruments, and assignments of carbon resonances were facilitated by DEPT experiments. Fast atom bombardment mass spectrometry (FABMS) to characterize synthetic peptides was carried out on a VG 707E-HF instrument, with glycerol or thioglycerol matrices being used to obtain both positive and negative ion spectra. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ.

Thin-layer chromatography was performed on Analtech or Merck silica gel GF plates (250 μm, 10 × 20 cm), and compounds were observed by fluorescence quenching and by spraying with a dilute ethanolic ninhydrin solution. Amino acid analyses and analytical HPLC were carried out on previously described Waters and Beckman instruments.

[[9-[(9-Fluorenylmethyloxycarbonyl)amino]xanthen-2-yl]oxy]acetic Acid [2-XAL$_1$] (2a). A solution of Fmoc-NH_2 (**14**) (0.91 g, 3.6 mmol) in glacial acetic acid (HOAc, 30 mL) was added to a solution of the xanthydrol intermediate **12a** (1.0 g, 3.3 mmol) in HOAc (55 mL). Next a solution of *p*-toluenesulfonic acid (0.1 g, 0.5 mmol) in HOAc (10 mL) was added over 20 min, and the reaction mixture was stirred for 24 h at 25 °C. The product precipitated slowly as a white solid, which was filtered, washed with H_2O (4 × 10 mL), and dried *in vacuo* over P_2O_5: yield 1.34 g (83%); mp 217−218 °C; TLC pure, R_f[acetone−EtOH−H_2O (12:1:1)] 0.54; ^{1}H NMR (CD_3SOCD_3) δ 8.38 (d, J = 8.8 Hz, NH), 7.89 (d, J = 7.3 Hz, 2H), 7.72 (d, J = 7.3 Hz, 2H), 7.35−7.5 (m, 6H), 7.1−7.2 (m, 3H), 6.93 (narrow m, 2H), 5.93 (d, J = 8.6 Hz, 1H), 4.66 (s, 2H), 4.40 (m, 2H), 4.25 (m, 1H).

Anal. Calcd for $C_{30}H_{23}NO_6$ (493.52): C, 73.00; H, 4.70; N, 2.84. Found: C, 73.06; H, 4.95; N, 3.01.

5-[[9-[(9-Fluorenylmethyloxycarbonyl)amino]xanthen-2-yl]oxy]valeric Acid [2-XAL$_4$] (2b). Method A. A suspension of xanthydrol intermediate **12b** (2.05 g, 6.5 mmol) and Fmoc-NH_2 (**14**) (1.46 g, 6.5 mmol) in glacial HOAc (50 mL) was heated to 50 °C, providing a clear yellow solution. A cottonlike white solid started to form after 10 min; the reaction was maintained for 1 h at 50 °C, and then the mixture was cooled to 25 °C. The light-beige product was collected by filtration, washed with H_2O (3 × 20 mL) and ethyl ether (3 × 20 mL), and dried *in vacuo* over P_2O_5 at 25 °C: yield 2.30 g (68%). **Method B.** Xanthydrylamine **15** (1.0 g, 3.2 mmol) was suspended in H_2O (100 mL), and Et_3N (0.82 g, 1.13 mL, 8.1 mmol, 2.5 equiv) was added to furnish a clear yellow solution. A solution of Fmoc-OSu (1.3 g, 3.85 mmol, 1.2 equiv) in CH_3CN (70 mL) was then added, and a gel-like precipitate started to form after 30 min. The reaction was maintained for a total of 3 h at 25 °C, and then HOAc was added to reach pH 3.3. The light-beige product was collected by filtration, washed with H_2O (3 × 50 mL) and CH_3CN (3 × 50 mL), and dried *in vacuo* over P_2O_5 at 25 °C: yield 1.55 g (91%); mp 210−211 °C; R_f[EtOAc−MeOH (5:1)] 0.53 (single spot); ^{1}H NMR (CD_3SOCD_3) δ 8.36 (d, J = 8.8 Hz, NH), 7.89 (d, J = 7.2 Hz, 2H), 7.71 (d, J = 7.2 Hz, 2H), 7.35−7.5 (m, 6H), 7.1−7.2 (m, 3H), 6.9−7.0 (m, 2H), 5.92 (d, J = 8.8 Hz, 1H), 4.42 (d, J = 6.8 Hz, 2H), 4.26 (t, J = 6.8 Hz, 1H), 3.93 (br t, 2H), 2.29 (t, J = 7.0 Hz, 2H), 1.6−1.8 (m, 4H).

Anal. Calcd for $C_{33}H_{29}NO_6$ (535.60): C, 74.00; H, 5.45; N, 2.61. Found: C, 73.85; H, 5.46; N, 2.45.

[[9-[(9-Fluorenylmethyloxycarbonyl)amino]xanthen-3-yl]oxy]acetic Acid [3-XAL$_1$] (3a). Method A. Following Caciagli *et al.*,[15] an amalgam was made from cut sodium (1.2 g, 53 mmol) and mercury (7.5 mL, 0.5 mol) which were warmed to 75 °C for 1 h. A suspension of keto acid **11a** (2.0 g, 7.4 mmol) in 95% EtOH (160 mL) was then added, and the white reaction mixture was heated to reflux for 2 h. The mixture was filtered while hot to remove residual amalgam and then concentrated to give a white solid which was dissolved in HOAc (120 mL) and combined with solid Fmoc-NH_2 (**14**) (3.3 g, 2 equiv). The brown reaction mixture was allowed to stand at 25 °C for 45 min (product began to precipitate after 15 min) and concentrated *in vacuo* to about a third to half of the original volume. The crude product was collected by filtration and purified by extraction with boiling EtOAc−MeOH (50 mL, 9:1 v/v). After cooling, the title light-beige solid was collected by filtration, washed with EtOAc (3 × 20 mL), and dried *in vacuo* over NaOH: yield 2.10 g (64%). **Method B (Preferred).** A mixture of keto acid **11a** (1.0 g, 3.7 mmol), NaOH (solid flakes) (0.74 g, 18 mmol), and activated zinc[37] (0.96 g, 15 mmol) was refluxed in 95% EtOH (100 mL) for 6 h to provide a light-pink solution. Zinc was removed by filtration; this was followed by washing with hot 95% EtOH (3 × 5 mL), and the combined filtrates were concentrated to give a light-pink solid which was dissolved in a mixture of TFA (2.3 mL, 30 mmol) and 1,2-dimethoxyethane (50 mL).[38] Fmoc-NH_2 (**14**) (1.1 g, 4.4 mmol) was added to this yellow mixture in one portion, and the resultant solution was stirred for 4 h at 25 °C. Solid K_2CO_3 (1.0 g, 7.4 mmol) was then added, and within 1 h at 25 °C, a white precipitate formed. The resultant potassium salt was collected by filtration, washed with EtOH (3 × 10 mL) to remove excess **14**, and then suspended in a mixture of H_2O−EtOH (50 mL, 5:1 v/v) and acidified to pH ~5 by addition of 1 M aqueous $KHSO_4$ (1.6 mL). The resultant light-beige solid was collected by filtration, washed with H_2O (3 × 10 mL), and dried *in vacuo* over P_2O_5: yield 1.32 g (73%); mp 170−172 °C; R_f[$CHCl_3$−MeOH−HOAc (10:1:0.1)] 0.53; ^{1}H NMR (CD_3SOCD_3) δ 8.25 (d, J = 8.8 Hz, NH), 7.87 (d, J = 7.2 Hz, 2H), 7.68 (d, J = 7.2 Hz, 2H), 7.10−7.41 (m, 9H), 6.68 (dd, J = 2.5 and 8.5 Hz, 1H), 6.57 (d, J = 2.5 Hz, 1H), 5.89 (d, J = 8.8 Hz, 1H), 4.50 (s, 2H), 4.37 (d, J = 6.5 Hz, 2H), 4.24 (t, J = 6.5 Hz, 1H).

Anal. Calcd for $C_{30}H_{23}NO_6$ (493.52): C, 73.00; H, 4.70; N, 2.84. Found: C, 72.98; H, 4.81; N, 2.77.

5-[[9-[(9-Fluorenylmethyloxycarbonyl)amino]xanthen-3-yl]oxy]valeric Acid [3-XAL$_4$] (3b). Method A. Compound **3b** was prepared essentially as reported for compound **3a**, but carried out starting with keto acid **11b** (0.50 g, 1.6 mmol). The crude product solid was purified by extraction with boiling EtOAc−hexane (15 mL, 1:1 v/v), since the use of EtOAc−MeOH gave a gel which was impossible to filter: yield 0.54 g (63%). **Method B (Preferred).** Compound **3b** was prepared again as reported for compound **3a**, starting with keto acid **11b** (1.0 g, 3.2 mmol): yield 1.33 g (77%); mp 120−122 °C; R_f[$CHCl_3$−MeOH−HOAc (10:1:0.1)] 0.55; ^{1}H NMR (CD_3SOCD_3) δ 8.27 (d, J = 8.8 Hz, NH), 7.89 (d, J = 7.2 Hz, 2H), 7.71 (d, J = 7.2 Hz, 2H), 7.14−7.42 (m, 9H), 6.71-6.76 (m, 2H), 5.89 (d, J = 8.8 Hz, 1H), 4.41 (d, J = 6.5 Hz, 2H), 4.24 (t, J = 6.5 Hz, 1H), 4.01 (t, J = 5.9 Hz, 2H), 2.31 (t, J = 6.8 Hz, 2H), 1.70 (m, 4H). Method B was carried out on a 0.2 mol scale at Hoffmann-La Roche, Inc., with comparable yields and purities.

Anal. Calcd for $C_{33}H_{29}NO_6$ (535.60): C, 73.99; H, 5.46; N, 2.62. Found: C, 73.80; H, 5.36; N, 2.69.

2-Hydroxyxanthone (4). A mixture of crude 2,2′,5-trimethoxybenzophenone (**6**) (32 g, 118 mmol) and pyridine

(37) It is strongly recommended to use *freshly* activated zinc, prepared as follows: zinc dust (2.0 g) was covered with 1.5 N aqueous HCl (20 mL) for ~5 min and then washed successively with H_2O (3 × 10 mL), EtOH (3 × 10 mL), acetone (3 × 5 mL), and ethyl ether (3 × 5 mL). The subsequent reaction was noted to fail sometimes when the zinc had been stored more than a day in a loosely sealed bottle, or when the reaction solvent was absolute EtOH instead of 95% EtOH as in the text experimental description.

(38) When the same reaction sequences were carried out using as solvent neat HOAc (compare to procedures for **2a** and **2b**) instead of DME containing TFA, the desired handles **3a** and **3b** could not be isolated. Rather, two phenomena took place: (i) the unstable intermediate xanthydrol (never isolated) disproportionated to starting ketone **11a** or **11b** and corresponding over-reduced xanthene **13a** or **13b** and (ii) Fmoc-NH_2 (**14**) was converted to dibenzofulvene R_f[hexane−EtOAc (10:1)] 0.56; ^{1}H NMR ($CDCl_3$) δ 7.74 (d, J = 7.2 Hz, 2H), 7.70 (d, J = 7.5 Hz, 2H), 7.38 (td, J = 0.9 and 7.2 Hz, 2H), 7.30 (td, J = 1.2 and 7.5 Hz, 2H), 6.08 (s, 2H) [matched signals from treatment of Fmoc-glycine with NH_3 in MeOH, quick evaporation, and redissolving in $CDCl_3$]; EIMS (solid probe, 70 eV, 25 °C) m/z 178.1 (calcd for $C_{14}H_{10}$, 178.08)].

hydrochloride (172 g, 1.5 mol) was heated to a gentle reflux (210 °C bath) under N_2. After 10 h of reaction, the mixture was quenched by pouring into ice H_2O (400 g), and the resultant yellow-green precipitate was collected, washed with H_2O (2 × 100 mL) and 6 N aqueous HCl (3 × 100 mL), and combined with hot 95% EtOH (50 mL) to form a suspension. The solid was collected again once the solvent had cooled, washed with 95% EtOH (2 × 25 mL), and dried *in vacuo*: yield: 20.6 g (82%); mp 245−246 °C [lit.[17a] mp 241 °C]; R_f[benzene−EtOH (20:3)] 0.54; ^{1}H NMR (CD_3SOCD_3) δ 10.0 (1H, OH), 8.17 (dd, J = 1.5 and 7.9 Hz, 1H), 7.85 (t, J = 7.7 Hz, 1H), 7.4−7.65 (m, 4H), 7.32 (dd, J = 3.1 and 9.0 Hz, 1H).

3-Hydroxyxanthone (5). Method A. Compound **5** was prepared essentially according to Van Allan,[17b] but with a simpler workup. A mixture of crude 2,2′,4-trimethoxybenzophenone (**7**) (26.4 g, 97 mmol) and pyridine hydrochloride (88.2 g, 0.76 mol) was heated (210 °C bath) for 4 h under N_2. The mixture was then poured into ice H_2O (200 g), and the yellow-green precipitate which formed was collected by filtration, washed with H_2O (2 × 100 mL) and 6 N aqueous HCl (3 × 100 mL), and dried *in vacuo*: yield 16.8 g (81%). **Method B.** A mixture of 2,2′-dihydroxy-4-methoxybenzophenone (10.0 g, 41 mmol) and pyridine hydrochloride (25.4 g, 0.22 mol) was heated (210 °C bath) for 6 h to form a melt at gentle reflux (210−215 °C). The mixture was then poured into ice H_2O (80 g), and the NMR-pure precipitate (8.9 g, quantitative) was recrystallized from hot 95% EtOH: yield 6.3 g (69%); mp 258−259 °C [lit.[17a] mp 246 °C]; R_f[benzene−EtOH (20:3)] 0.6; ^{1}H NMR (CD_3SOCD_3) δ 11.0 (br s, OH), 8.12 (dd, J = 1.6 and 7.9 Hz, 1H), 8.02 (d, J = 8.2 Hz, 1H), 7.77 (td, J = 1.7 and 7.9 Hz, 1H), 7.63 (d, J = 8.2 Hz, 1H), 7.46 (td, J = 1.1 and 7.9 Hz, 1H), 6.92 (dd, J = 2.1 and 8.2 Hz, 1H), 6.89 (d, J = 2.1 Hz, 1H).

Anal. Calcd for $C_{13}H_8O_3$ (212.20): C, 73.58; H, 3.80. Found: C, 73.61; H, 3.83.

2,2′,5-Trimethoxybenzophenone (6). Method A. A mixture of *o*-anisoyl chloride (21.8 mL, 0.147 mol) and 1,4-dimethoxybenzene (37.2 g, 0.27 mol) was heated under N_2 for 20 h (200 °C bath). Distillation after HCl evolution subsided led to recovered 1,4-dimethoxybenzene [18.7 g, bp 92−95 °C (8 mm), ^{1}H NMR ($CDCl_3$) δ 6.85 (s, 4H), 3.77 (s, 3H)], followed by the title product at bp 232 °C (7 mm) [lit.[17c] bp 237−239 °C (18 mm)], a viscous yellow oil which was suitable for carrying forward to the next step: yield 25.4 g (63%). **Method B.** A mixture of *o*-anisoyl chloride (39 mL, 0.26 mol) and carbon disulfide (53 mL) was added dropwise over 2 h under ambient conditions to a stirred mixture of 1,4-dimethoxybenzene (75 g, 0.54 mol, soluble) and anhydrous aluminum trichloride (46 g, 0.35 mol, suspended) in carbon disulfide (95 mL). Reaction was accompanied by vigorous HCl evolution and a modest spontaneous exotherm. After 4 h, the reaction was quenched by pouring the solution into a mixture of ice (1 kg) and 12 N aqueous HCl (10 mL). The organic layer was washed with aqueous saturated NaCl (1 L), H_2O (2 × 1 L), and again with aqueous saturated NaCl (1 L), dried (Na_2SO_4), and distilled to give recovered 1,4-dimethoxybenzene (40 g), followed by the title product: yield 61 g (85%); ^{1}H NMR ($CDCl_3$) δ 7.35−7.51 (m, 2H), 6.8−7.1 (m, 5H), 3.78 (s, 3H), 3.68 (s, 3H), 3.57 (s, 3H).

2,2′,4-Trimethoxybenzophenone (7). Following Van Allan,[17b] a mixture of *o*-anisoyl chloride (21.8 mL, 0.147 mol) and 1,3-dimethoxybenzene (35.7 mL, 0.27 mol) was heated under N_2 for 3 h (200 °C bath). Distillation, after HCl evolution subsided, led to recovered 1,3-dimethoxybenzene [20 g; bp 75−80 °C (5 mm)], followed by title product, bp 241−243 °C (6 mm) [lit.[17b] bp 180−200 °C (15 mm); this literature value is likely an underestimate of the true value], in adequate purity for carrying forward to the next step: yield 34 g (86%); R_f[benzene−EtOH (20:3)] 0.9; ^{1}H NMR ($CDCl_3$) δ 7.59 (d, J = 8.6 Hz, 1H), 7.35−7.45 (m, 2H), 6.9−7.1 (m, 2H), 6.45−6.55 (m, 2H), 3.85 (s, 3H), 3.69 (s, 3H), 3.65 (s, 3H).

Ethyl [(9-Oxoxanthen-2-yl)oxy]acetate (8a). A mixture of 2-hydroxyxanthone (**4**) (14.1 g, 66 mmol), ethyl bromoacetate (14.5 mL, 0.13 mol), and anhydrous K_2CO_3 (53 g, 0.38 mol) in acetone (350 mL) plus DMF (12 mL) was refluxed for 6 h. The cooled reaction mixture was filtered to remove inorganic salts, washed with acetone (3 × 50 mL) and ether (3 × 50 mL), concentrated, and placed under hexane, whereupon a powdery brown solid formed: yield 16.1 g (82%); mp 91−92 °C; R_f[benzene−EtOH (20:3)] 0.81 (one spot); ^{1}H NMR (CD_3SOCD_3) δ 8.19 (dd, J = 1.6 and 7.9 Hz, 1H), 7.86 (td, J = 1.7 and 7.8 Hz, 1H), 7.42−7.67 (m, 5H), 4.94 (s, 2H), 4.20 (q, J = 7.1 Hz, 2H), 1.32 (t, J = 7.1 Hz, 3H). An analytical sample from *n*-hexane−EtOH (1:1) was fine light-beige needles, mp 96−98 °C [lit.[15,18a] mp 97−98 °C].

Anal. Calcd for $C_{17}H_{14}O_5$ (298.28): C, 68.45; H, 4.73. Found: C, 68.65; H, 5.01.

Ethyl 5-[(9-Oxoxanthen-2-yl)oxy]valerate (8b). Potassium *tert*-butoxide (4.6 g, 41 mmol) was added in one portion to a solution of 2-hydroxyxanthone (**4**) (7.7 g, 36 mmol) in DMF (50 mL). The resultant suspension (supernatant dark red) was stirred briefly at 25 °C under N_2, and then a solution of ethyl 5-bromovalerate (7.1 mL, 42 mmol) in DMF (20 mL) was added dropwise over 20 min. The reaction mixture was heated at for 11 h at 115 °C and then cooled, filtered, and washed with EtOAc (2 × 10 mL). The filtrate was concentrated to provide a light-brown oily residue, which solidified after standing overnight as the neat oil or under hexane. Light-beige crystals were collected and washed with *n*-hexane (3 × 10 mL): yield 9.7 g (79%); mp 59−61 °C; R_f[benzene−EtOH (10:9)] 0.79; ^{1}H NMR (CD_3SOCD_3) δ 8.22 (dd, J = 1.6 and 8.0 Hz, 1H), 7.89 (td, J = 1.6 and 7.5 Hz, 1H), 7.4−7.7 (m, 5H), 4.14 (q, J = 7.1 Hz, 2H), 4.09 (t, J = 5.7 Hz, 2H), 2.40 (t, J = 6.9 Hz, 2H), 1.85 (m, 4H), 1.26 (t, J = 7.1 Hz, 3H). An analytical sample from *n*-hexane−EtOH (10:1) was white needles, mp 58−59 °C.

Anal. Calcd for $C_{20}H_{20}O_5$ (340.36): C, 70.57; H, 5.92. Found: C, 70.52; H, 5.86.

Ethyl [(9-Oxoxanthen-3-yl)oxy]acetate (9a). As described by Puranik *et al.*,[18b] a mixture of 3-hydroxyxanthone (**5**) (6.0 g, 28 mmol), ethyl bromoacetate (7.7 mL, 69 mmol), and anhydrous K_2CO_3 (25.4 g, 0.18 mol) in dry acetone (500 mL) was refluxed for 12 h and then filtered and concentrated: yield 7.4 g (88%). Crystallization from EtOH gave the title product as shiny colorless needles: mp 122−123 °C [lit.[15,18a] mp 122−124 °C; lit.[18b] mp 135−136 °C]; R_f[hexane−EtOH (10:3)] 0.44; ^{1}H NMR ($CDCl_3$) δ 8.32 (dd, J = 1.6 and 7.8 Hz, 1H), 8.27 (d, J = 8.9 Hz, 1H), 7.69 [td, J = 1.6 and 7.5 Hz, 1H], 7.45 (d, J = 7.8 Hz, 1H), 7.36 (td, J = 1.0 and 7.5 Hz, 1H), 6.98 (dd, J = 2.4 and 8.9 Hz, 1H), 6.87 (d, J = 2.4 Hz, 1H), 4.74 (s, 2H), 4.30 (q, J = 7.1 Hz, 2H), 1.31 (t, J = 7.1 Hz, 3H).

Anal. Calcd for $C_{17}H_{14}O_5$ (298.28): C, 68.45; H, 4.73. Found: C, 68.65; H, 5.01.

Ethyl 5-[(9-Oxoxanthen-3-yl)oxy]valerate (9b). Anhydrous K_2CO_3 (51.3 g, 372 mmol) was added to a solution of 3-hydroxyxanthone (**5**) (20.5 g, 97 mmol) plus ethyl 5-bromovalerate (53.3 g, 316 mmol) in dry acetone (1.5 L).[39] The reaction mixture was heated to reflux for 8 h, cooled, filtered to remove inorganic salts, washed with acetone (3 × 50 mL), and concentrated. The initial brownish crystals were washed with hexane (3 × 60 mL), providing white crystals which were dried *in vacuo* over P_2O_5: yield 28.5 g (86%); mp 75−77 °C; R_f[hexane−EtOH (10:3)] 0.51; ^{1}H NMR ($CDCl_3$) δ 8.31 (dd, J = 1.6 and 7.9 Hz, 1H), 8.23 (d, J = 8.8 Hz, 1H), 7.67 (td, J = 1.6 and 7.0 Hz, 1H), 7.44 (d, J = 7.9 Hz, 1H), 7.35 (td, J = 1.0 and 7.5 Hz, 1H), 6.92 (dd, J = 2.3 and 8.8 Hz, 1H), 6.84 (d, J = 2.3 Hz, 1H), 4.06−4.19 (m, 4H), 2.41 (t, J = 6.8 Hz, 2H), 1.82−1.89 (m, 4H), 1.26 (t, J = 7.1 Hz, 3H).

Anal. Calcd for $C_{20}H_{20}O_5$ (340.36): C, 70.59; H, 5.92. Found: C, 70.51; H, 5.83.

[(9-Oxoxanthen-2-yl)oxy]acetic Acid, Potassium Salt and Free Acid (10a). Ester **8a** (10 g, 33 mmol) was dissolved in 95% EtOH (150 mL), and 4 N aqueous KOH (30 mL, 0.12 mol) and H_2O (15 mL) were added. The mixture was stirred for 30 min at 35 to 40 °C and then cooled, and the solid precipitate which formed was collected by filtration, washed with absolute ether (3 × 20 mL), and air-dried: yield 9.25 g (96%); R_f[MeOH−H_2O (4:1)] 0.90; ^{1}H NMR (CD_3SOCD_3) δ 8.18

(39) The text procedure using anhydrous K_2CO_3 gave much better yields and purities than when potassium *tert*-butoxide was used as base (compare to procedure for **8b**).

(dd, J = 1.6 and 7.9 Hz, 1H), 7.86 (td, J = 1.6 and 7.9 Hz, 1H), 7.35−7.65 (m, 5H), 4.19 (s, 2H). The potassium salt isolated in this way was used directly in the next reaction. A small portion was converted to the free acid by dissolving in H_2O and acidification with 12 N aqueous HCl to pH ∼1, providing a quite insoluble light-beige solid, mp 179−182 °C [lit.[18a] 180−181 °C]. ^{1}H NMR [DCON$(CD_3)_2$] aromatic region essentially the same (less fine structure) as that described above for the salt, CH_2 shifted to δ 4.94, carboxyl H at δ 13.5 (br).

5-[(9-Oxoxanthen-2-yl)oxy]valeric Acid (10b). A suspension of ethyl ester **8b** (6.8 g, 20 mmol) in 95% EtOH (50 mL) was diluted with 4 N aqueous NaOH (50 mL, 0.2 mol). After 1 h at 25 °C, the reaction mixture was homogeneous; saponification was quenched after 4 h by the addition with cooling of 12 N aqueous HCl (17 mL) to adjust the pH to 3. A light-gray precipitate formed quickly, which was collected, washed with H_2O (3 × 10 mL), and dried *in vacuo* over P_2O_5 to give the title product (free acid): yield 6.1 g (94%); mp 151−152 °C; ^{1}H NMR (CD_3SOCD_3) δ 8.20 (dd, J = 1.6 and 8.0 Hz, 1H), 7.87 (td, J = 1.6 and 7.5 Hz, 1H), 7.45−7.7 (m, 5H), 4.09 (t, J = 5.9 Hz, 2H), 2.31 (t, J = 7.1 Hz, 2H), 1.6−1.9 (m, 4H). An analytical sample from *n*-hexane−EtOH (10:1) was fine light-beige needles, mp unchanged.

Anal. Calcd for $C_{18}H_{16}O_5$ (312.31): C, 69.21; H, 5.16. Found: C, 69.15; H, 5.01.

[(9-Oxoxanthen-3-yl)oxy]acetic Acid, Free Acid and Potassium Salt (11a). Ester **9a** (3.0 g, 10 mmol) was dissolved in 95% EtOH (50 mL) at reflux, and 4 N aqueous KOH (50 mL, 0.2 mol) was added. After 2 h reflux, the alcohol was evaporated *in vacuo*, H_2O (100 mL) was added, and the mixture was brought to boiling to dissolve the potassium salt of the title product. The hot solution was filtered and acidified with 12 N aqueous HCl while still warm. The title product (free acid) came out of solution and was collected by filtration: yield 2.4 g (89%); recrystallized from EtOH to provide shiny colorless needles, mp 199−201 °C [lit.[15,18a,c] mp 196−198 °C; lit.[18b] mp 204 °C]; R_f[$CHCl_3$−MeOH−HOAc (10:1:0.1)] 0.57; ^{1}H NMR (CD_3SOCD_3) δ 8.19 (dd, J = 1.6 and 7.9 Hz, 1H), 8.13 (d, J = 8.8 Hz, 1H), 7.87 (td, J = 1.6 and 7.1 Hz, 1H), 7.65 (d, J = 8.3 Hz, 1H), 7.49 (td, J = 1.1 and 7.8 Hz, 1H), 7.16 (d, J = 2.4 Hz, 1H), 7.10 (dd, J = 2.4 and 8.8 Hz, 1H), 4.94 (s, 2H).

Anal. Calcd for $C_{15}H_{10}O_5$ (270.23): C, 66.67; H, 3.73. Found: C, 66.65; H, 3.76.

Alternatively, upon completion of the reflux, more alcohol (40 mL) was added, and after cooling, the potassium salt of the title product was collected: yield 2.8 g (91%).

Anal. Calcd for $C_{15}H_9O_5K$ (308.32): C, 58.43; H, 2.94. Found: C, 58.45; H, 2.89.

5-[(9-Oxoxanthen-3-yl)oxy]valeric Acid (11b). Ethyl ester **9b** (28.5 g, 84 mmol) was suspended in a mixture of 95% EtOH (210 mL) and 4 N aqueous NaOH (210 mL); a yellow homogeneous solution was obtained within 30 min and stirring continued at 25 °C for a total of 4 h. Partial evaporation to remove EtOH was followed by addition of H_2O (200 mL), followed by 6 N aqueous HCl under cooling to bring the pH to 3.5. The resultant white precipitate was collected by filtration, washed with H_2O (3 × 50 mL), and dried *in vacuo* over P_2O_5: yield 26.0 g (99%); mp 201−202 °C [lit.[18c] mp 191−193 °C]; R_f[EtOAc−hexane (10:3)] 0.48; ^{1}H NMR (CD_3SOCD_3) δ 8.18 (dd, J = 1.6 and 7.9 Hz, 1H), 8.09 (d, J = 8.9 Hz, 1H), 7.83 (td, J = 1.6 and 7.1 Hz, 1H), 7.62 (d, J = 7.9 Hz, 1H), 7.47 (t, J = 7.5 Hz, 1H), 7.15 (d, J = 2.3 Hz, 1H), 7.04 (dd, J = 2.3 and 8.8 Hz, 1H), 4.17 (t, J = 6.0 Hz, 2H), 2.32 (t, J = 7.0 Hz, 2H), 1.71 (m, 4H).

Anal. Calcd for $C_{18}H_{16}O_5$ (312.31): C, 69.23; H, 5.16. Found: C, 69.47; H, 5.26.

[(9-Hydroxyxanthen-2-yl)oxy]acetate, Mixture of Sodium and Potassium Salts (12a). A solution of potassium salt **10a** (8.48 g, 27.5 mmol) in H_2O (70 mL) was treated with $NaBH_4$ (2.0 g, 52.8 mmol) which was added in small portions over 1.5 h while stirring. After 20 h at 25 °C, further $NaBH_4$ (1.0 g, 26.4 mmol) was added, and reduction was continued for 26 h at 25 °C. The resultant white precipitate was filtered, washed with EtOH (3 × 50 mL), combined with a second crop which appeared after partial concentration of the mother liquor, and air-dried:[40] yield 7.2 g (89%); R_f[EtOH−H_2O−EtOAc (12:1:1)] 0.22 (major spot which became yellow after spraying with 2% TFA in CH_2Cl_2); ^{1}H NMR (CD_3SOCD_3) δ 7.52 (d, J = 8.0 Hz, 1H), 7.0−7.35 (m, 5H), 6.74 (dd, J = 3.0 and 8.0 Hz, 1 Hz), 6.2 (s, 1H, exchanges with D_2O), 5.61 (s, 1H), 4.12 (s, 2H).

5-[(9-Hydroxyxanthen-2-yl)oxy]valeric Acid (12b). A solution of 1 N aqueous NaOH (5.5 mL, 5.5 mmol) was added dropwise to dissolve completely keto acid **10b** (1.5 g, 4.8 mmol) in H_2O (36 mL); the final pH of the yellow solution was 8.3. Subsequently, $NaBH_4$ (1.5 g, 39 mmol) was added in a single portion, and the reaction mixture was stirred for 2.5 h at 50−55 °C.[41a] The pale yellow solution was cooled to 10 °C, and solid $NaHCO_3$ (2.7 g, 10 equiv) was added in small portions over 30 min.[41b] After maintenance at 25 °C for 1 h, the solution was cooled externally to <5 °C, and a mixture of HOAc (10 mL) and ice H_2O (10 g) was added [final pH ∼5.5]. A fine milky white precipitate was collected by filtration, washed with H_2O (3 × 30 mL), and dried *in vacuo* over P_2O_5: yield 1.4 g (93%); mp 92−94 °C; R_f[EtOAc−MeOH (2:1)] 0.51; ^{1}H NMR (CD_3SOCD_3) δ 7.61 (d, J = 6.2 Hz, 1H), 7.32 (t, J = 6.8 Hz, 1H), 7.0−7.2 (m, 4H), 6.92 (dd, J = 3.0 and 8.9 Hz, 1H), 5.73 (s, 1H), 4.04 (t, J = 6.0 Hz, 2H), 2.32 (t, J = 6.9 Hz, 2H), 1.7−1.9 (m, 4H); title product[41c] suitable for direct use in next reaction.

(Xanthen-3-yloxy)acetic Acid (13a). Substrate **11a** (0.5 mmol scale) was treated with $NaBH_4$ under the same conditions as for the reduction of the 2-xanthenyl analogue **10a** successfully to the corresponding xanthydrol **12a**. Instead of the expected xanthydrol, the NMR-pure title product was isolated in 70% yield: mp 172−173 °C; R_f[CH_3Cl−MeOH−HOAc (10:1:0.1)] 0.67; ^{1}H NMR (CD_3SOCD_3) δ 7.04−7.23 (m, 5H), 6.62 (dd, J = 2.4 and 8.4 Hz, 1H), 6.55 (d, J = 2.4 Hz, 1H), 4.48 (S, 2H), 3.96 (s, 2H).

Anal. Calcd for $C_{15}H_{12}O_4$ (256.07): C, 70.29; H, 4.72. Found: C, 70.11; H, 4.53.

5-(Xanthen-3-yloxy)valeric Acid (13b). Compound **13b** was prepared similarly to compound **13a**, but starting from substrate **11b** (0.5 mmol scale) (68% yield): mp 133−135 °C; R_f[EtOAc−hexane (10:3)] 0.63; ^{1}H NMR (CD_3SOCD_3) δ 7.03−7.27 (m, 5H), 6.65−6.68 (m, 2H), 3.97 (m, 4H), 2.29 (t, J = 6.8 Hz, 2H), 1.69 (m, 4H).

9-Fluorenylmethyl Carbamate (14). Following Stüber *et al.*,[8c] 1.5 N aqueous NH_4OH (60 mL, 90 mmol) was added dropwise with stirring over 10 min to a solution of 9-fluorenylmethyl chloroformate (10.3 g, 40 mmol) in 1,2-dimethoxyethane−H_2O (4:1, 100 mL). As the ammonia was added, the title product came out of solution. The reaction endpoint was indicated by a pH of 7.4, which was maintained for 30 min, followed by acidification to a pH of 6.5 with 1.0 N HCl (∼1.5 mL). The reaction mixture was chilled in ice, and a white fluffy solid was collected by filtration, followed by careful washing with cold H_2O (3 × 10 mL) and drying *in vacuo* over P_2O_5: yield 9.4 g (99%); mp 207−208 °C [lit.[8c] mp 205−206

(40) The workup is critical. When this reaction mixture was quenched with either 10% aqueous HOAc or 10% aqueous citric acid, over-reduced byproduct (xanthenyl-2-oxy)acetic acid was formed (5% by HPLC). Characterization after isolation by column chromatography: mp 193−194 °C; ^{1}H NMR (CD_3SOCD_3) δ 7.23 (d, J = 7.0 Hz, 1H), 7.19 (d, J = 7.5 Hz. 1H), 6.98−7.05 (m, 3H), 6.81 (d, J = 2.5 Hz, 1H), 6.78 (dd, J = 2.5 and 8.5 Hz, 1H), 4.63 (s, 2H), 4.00 (s, 2H). Anal. Calcd for $C_{15}H_{12}O_4$ (256.07): C, 70.29; H, 4.72. Found: C, 70.19; H, 4.68.

(41) (a) Omission of base was accompanied by uncontrollable foaming, whereas a higher initial pH (i.e., 11−12) significantly retarded the reaction rate. (b) This step was found to be critical. Quenching directly into HOAc−ice water was invariably accompanied by the formation of over-reduced byproduct: 5-(xanthenyl-2-oxy)valeric acid, mp 149−150 °C; TLC, R_f[EtOAc−MeOH (2:1)] 0.84, [EtOAc−hexane (10:3)] 0.62; ^{1}H NMR (CD_3SOCD_3) δ 7.22−7.26 (m, 2H), 6.98−7.08 (m, 3H), 6.78−6.82 (m, 2H), 4.02 (s, 2H), 3.94 (t, J = 5.7 Hz, 2H), 2.29 (t, J = 6.5 Hz, 2H), 1.67 (m, 4H). Anal. Calcd for $C_{18}H_{18}O_4$ (298.12): C, 72.45; H, 6.09. Found: C, 72.28; H, 6.24. An authentic sample of this over-reduced material was prepared in excellent yield and purity by quenching the title reaction mixture [which contains xanthydrol **12b**] with 5% aqueous TFA]. (c) The reduced byproduct was absent, as judged by ^{1}H NMR.

°C]; R_f[benzene−EtOH(10:3)] 0.74; ^{1}H NMR (CD_3SOCD_3) δ 7.90 (d, J = 6.8 Hz, 2H), 7.70 (d, J = 7.0 Hz, 2H), 7.43 (t, J = 7.2 Hz, 2H), 7.34 (t, J = 7.3 Hz, 2H), 6.6 (br, 2H, NH_2), 4.21−4.29 (m, 3H); ^{1}H NMR ($CDCl_3$) δ 7.76 (d, J = 6.9 Hz, 2H), 7.60 (d, J = 7.1, 2H), 7.25−7.40 (m, 4H), 4.71 (br, 2H, NH_2), 4.40 (d, J = 6.9, 2H, CH_2), 4.23 (t, J = 6.9 Hz, 1H, CH).

Anal. Calcd for $C_{15}H_{13}NO_2$ (239.27): C, 75.30; H, 5.48; N, 5.85. Found: C, 75.37; H, 5.60; N, 5.82.

5-[(9-Aminoxanthen-2-yl)oxy]valeric Acid (15). Starting with keto acid **10b** (2.0 g, 6.4 mmol), $NaBH_4$ reduction was carried out exactly as in the procedure for generating xanthydrol **12b**. Upon completion of the $NaHCO_3$ quenching step, $(NH_4)HCO_3$ (15.2 g, 30 equiv) was added for a 24 h reaction at 25 °C. The resultant white suspension was acidified to pH ∼4.5 by dropwise addition of 10% aqueous citric acid, providing a light-beige product which was collected by filtration, washed with H_2O (3 × 20 mL), and dried *in vacuo* over P_2O_5: yield 1.87 g (94%); mp 122−124 °C; R_f[$CHCl_3$−MeOH−HOAc (85:15:3)] 0.38 (single bright spot under UV); ^{1}H NMR (CD_3SOCD_3) δ 7.62 (d, J = 7.8 Hz, 1H), 7.01−7.31 (m, 5H), 6.85 (dd, J = 2.8 and 8.9 Hz, 1H), 4.94 (s, 1H), 3.97 (t, J = 5.9 Hz, 2H), 2.26 (t, J = 6.8 Hz, 2H), 1.7−1.9 (m, 4H).

Anal. Calcd for $C_{18}H_{19}NO_4$ (313.18): C, 69.01; H, 6.07; N, 4.47. Found: C, 69.28; H, 6.16; N, 4.27.

XAL-resins and Their Use in Solid-Phase Peptide Synthesis. Fmoc-XAL (**2a,b** and **3a,b**) were introduced readily onto a variety of amino-functionalized supports. The parent supports used were either 4-methylbenzhydrylamino-poly(styrene-co-1% divinylbenzene) (MBHA-PS) resin [PerSeptive Biosystems (formerly Millipore), initial loading 0.30 or 0.46 mmol/g)] or several polyethylene glycol-polystyrene (PEG-PS) graft supports (initial loadings 0.10−0.25 mmol/g) developed in our laboratory and commercially available from PerSeptive Biosystems; these latter are particularly well suited for continuous-flow synthesis.[22a] In general, a norleucine "internal reference" amino acid residue,[6d,42] with its N^{α}-amino function protected by Boc or Fmoc, was introduced first onto the support (standard DIPCDI/HOBt in DMF protocol, Table 3), following which the Boc or Fmoc group was removed in the usual way and the handle was added. In a typical procedure, handle 3-XAL_4 (**3b**) (278 mg, 0.52 mmol, 1.3 equiv), *N*-[(dimethylamino)(1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) (198 mg, 0.52 mmol, 1.3 equiv), and *N*,*N*-diisopropylethylamine (DIEA) (182 μL, 1.04 mmol, 2.6 equiv) were dissolved in *N*,*N*-dimethylformamide (DMF) (3 mL) and added to PEG-PS support (2.0 g, 0.2 mmol/g, 0.4 mmol, 1.0 equiv) which had just been subjected to a final wash and filtration with DMF. The coupling was allowed to proceed in a standard manual solid-phase reaction vessel by shaking at 25 °C for 16 h, at which point a qualitative ninhydrin test[43] indicated that the reaction had gone to completion. The resin was filtered, washed with DMF, CH_2Cl_2, and MeOH (3 × 5 mL × 1 min per solvent), and then dried *in vacuo* over P_2O_5. Fmoc-XAL-resins were extended further by stepwise automated Fmoc chemistry with standard HATU/DIEA, DIPCDI/HOBt or HOAt, or BOP/HOBt/NMM protocols (Tables 2, 3, and 4). Removal of the Fmoc group from XAL-resins occurred under the same conditions as Fmoc removal from resin-bound N^{α}-amino groups. Incorporations of amino acids occurred at the expected integer ratios. In fact, the "internal reference" amino acid technique showed that with optimal protocols, nearly quantitative yields were obtained for all steps, including introduction of the handle, removal of the handle Fmoc group, and stepwise incorporation of Fmoc-protected amino acid derivatives. Upon completion of peptide chain assemblies, aliquots (10−15 mg) of peptide-resin were treated with a variety of TFA/scavenger/CH_2Cl_2 cleavage cocktails (1 mL). Unless specified otherwise, initial cleavage from the support was carried out for 1 h at 25 °C, following which the filtrate was diluted with an equal volume of an "inverse" cocktail designed to bring the final concentration of TFA to 50% while maintaining the designated scavenger concentration. After a further 1 h at 25 °C, cleavages were quenched by dilution with HOAc−H_2O (4 mL, 3:7), and the CH_2Cl_2 layers were removed. The aqueous layers were extracted further with CH_2Cl_2 (5 × 1 mL) to remove scavengers, then purged with N_2 to remove residual CH_2Cl_2, and finally lyophilized to provide crude peptide for HPLC analysis. Cleavage yields were calculated on the basis of the amino acid compositions (with respect to Nle "internal reference") of the peptide-resins before and after cleavage.

Table 2. Automated Deprotection/Coupling Cycle Using *N*-[(Dimethylamino)(1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl)-methylene]-*N*-methylmethanaminium Hexafluorophosphate *N*-Oxide (HATU)/*N*,*N*-Diisopropylethylamine (DIEA) or *N*,*N*′-Diisopropylcarbodiimide (DIPCDI)/1-Hydroxybenzotriazole (HOBt) or 7-Aza-1-hydroxybenzotriazole (HOAt)[a]

operation	reagent, solvent	min
1	DMF washes	1
2	piperidine−DMF (1:4)	6
3	DMF washes	12
4a	AA/HATU/DIEA (1:1:2) in DMF[b,c]	30
4b	AA/DIPCDI/HOBt or HOAt (1:1:1) in DMF[b,d]	30
5	DMF washes	8

[a] These operations are performed automatically in the continuous-flow mode on a PerSeptive Biosystems 9050 peptide synthesizer. [b] The indicated reagents were used in 4-fold excess over growing chains on the peptide-PEG-PS supports. [c] The appropriate N^{α}-Fmoc-protected amino acid and the HATU reagent were combined together as solids in the same instrument vial and were dissolved approximately 6 min before needed to a final concentration of 0.3 M by automated addition of DIEA (0.6 M) in DMF. [d] Similar to previous the note, the appropriate solid N^{α}-Fmoc-protected amino acid and the HOAt or HOBt reagent were dissolved approximately 6 min before needed to a final concentration of 0.3 M by addition of DIPCDI (0.3 M) in DMF.

Table 3. Deprotection/Coupling Cycle Using *N*,*N*′-Diisopropylcarbodiimide (DIPCDI)/1-Hydroxybenzotriazole (HOBt) or 7-Aza-1-hydroxybenzotriazole (HOAt)[a]

operation	reagent, solvent	time, min
1	DMF washes	1 × 0.3
2	piperidine−DMF (1:4)	1 × 1 + 2 × 3
3	DMF washes	5 × 0.3
4	AA/DIPCDI/HOBt or HOAt (1:1:1) in DMF[b,c]	30[d]
5	DMF washes	4 × 0.3

[a] Protocol for manual operation. [b] The indicated reagents were used in 4-fold excess over growing chains on the peptide-resin. [c] HOBt or HOAt were dissolved in DMF (final concentration ∼0.3 M), and these solutions were used to dissolve the appropriate N^{α}-Fmoc-protected amino acids. The resultant DMF solutions were then added to the deprotected peptide-resin, followed by addition of neat DIPCDI. [d] Qualitative ninhydrin tests (ref 43) performed at this point verified that the coupling reactions had gone to completion.

Leu-Ala-Gly-Val-NH_2. Kinetic Studies. Fmoc-XAL (**2a,b** and **3a,b**) were linked to a Phe-PEG-PS support (100 mg per experiment, 0.21 mmol/g), which comprised PEG:PS in an approximately 1:1 ratio. Automatic continuous-flow solid-phase synthesis with Fmoc-amino acids and an HATU/DIEA protocol (Table 2) provided the appropriate peptide-resins with satisfactory amino acid analyses for all syntheses (Leu, Ala, Gly, and Val all 1.00 ± 0.02). The peptide-resins were treated with TFA−CH_2Cl_2 (1:19), and samples (∼5 mg) were withdrawn periodically (abscissa of Figure 1A), washed with CH_2Cl_2 (5 × 1 mL), and hydrolyzed in order to determine cleavage yields (ordinate of Figure 1A). Additionally peptide-resin derived from 3-XAL_4 (**3b**) was cleaved with TFA−CH_2Cl_2 (1:99) and (1:49), as well as (1:19) (Figure 1B). The cleaved tetrapeptide amide from these syntheses was of >99% purity by HPLC: Delta Pak C_{18} reversed-phase column (3.9

(42) (a) Atherton, E.; Clive, D. L.; Sheppard, R. C. *J. Am. Chem. Soc.* **1975**, *97*, 6584−6585. (b) Matsueda, G. R.; Haber, E. *Anal. Biochem.* **1980**, *104*, 215−227.

(43) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. *Anal. Biochem.* **1970**, *34*, 595−598.

Table 4. Automated Deprotection/Coupling Cycle Using (Benzotriazolyloxy)tris(dimethylamino)phosphonium Hexafluorophosphate (BOP)/1-Hydroxybenzotriazole (HOBt)/*N*-Methylmorpholine (NMM) or *N,N′*-Diisopropylcarbodiimide (DIPCDI)/ 1-Hydroxybenzotriazole (HOBt)[a,b]

operation	reagent, solvent	time, min
1	CH_2Cl_2−DMF (1:1) washes	10 × 0.3
2	piperidine−DMF−toluene (6:7:7)	5 + 8
3	CH_2Cl_2−DMF (1:1) washes	12
4a	AA/BOP/HOBt/NMM (1:1:1:1) in DMF[c]	60
4b	AA/DIPCDI/HOBt (1:1:1) in DMF[d]	60

[a] These operations are performed automatically in a batchwise mode on a PerSeptive Biosystems 9600 peptide synthesizer. [b] This protocol can also be used substituting (7-azabenzotriazolyloxy)-tris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP) for BOP. [c] The indicated equimolar reagents were used in 8-fold excess over growing chains on the peptide-resin. The appropriate N^{α}-Fmoc-protected amino acid, BOP reagent, and HOBt were stored as solids in the reservoirs of the instrument; they were dissolved and activated by addition or equimolar NMM (0.2 M) in DMF. After 2 min at 25 °C, this homogeneous "preactivated" mixture was added directly to the deprotected peptide-resin. [d] The indicated equimolar reagents were used in 17-fold excess over growing chains on the peptide-resin [this seemingly high excess reflects the relatively low initial loading for experiments in which this particular protocol was used]. The appropriate N^{α}-Fmoc-protected amino acid, DIPCDI reagent, and HOBt were stored as solids in the reservoirs of the instrument; DMF was added when needed and after 2 min at 25 °C, a homogeneous "preactivated" mixture was added directly to the deprotected peptide-resin.

× 150 mm, 5 μm), linear gradient over 20 min of 0.036% TFA in CH_3CN and 0.045% aqueous TFA, from 1:9 to 9:1, flow rate 1.0 mL/min, t_R 18.6 min.

Fmoc-Ala-Pro-Trp-Ala-Val-Leu-Glu(O^{t}Bu)-Val-Ala-NH_2. Fmoc-3-XAL_4 (**3b**) was linked to a PEG-PS support (300 mg, 0.21 mmol/g), which comprised PEG:PS in an approximately 1:1 ratio. Couplings of Fmoc-amino acids were performed automatically on a continuous-flow PerSeptive Biosystems 9050 peptide synthesizer using a HATU/DIEA coupling protocol (Table 2, operation 4a). The side-chain carboxyl of glutamic acid was protected as the *tert*-butyl ester, and other residues were unprotected. The peptide-resin was preswollen with washes of CH_2Cl_2 (3 mL, 3 × 5 min), and the cleavage was performed by alternating washes of TFA−CH_2Cl_2 (1:99) (3 mL, 6 × 5 min) and CH_2Cl_2 (3 mL, 5 × 0.5 min). The combined filtrates and washes were concentrated by rotary evaporation. The yield of cleavage was 84%, and the amino acid composition of the crude cleaved peptide was Glu, 1.01; Ala, 2.89; Pro, 1.02; Val, 2.04; Leu, 1.04. HPLC analysis (Figure 2) indicated >90% purity.

***T. atratus* Adipokinetic Hormone (pGlu-Leu-Thr-Phe-Thr-Pro-Gly-Trp-NH_2).**[6b,29] **Method A.** Fmoc-2-XAL_4 (**2b**) was linked to a PEG-PS support (100 mg, 0.1 mmol/g) prepared by Dr. Jane L. Chang, which comprised PEG:PS in an approximately 1:1 ratio. Couplings of Fmoc-amino acids were performed automatically on a continuous-flow PerSeptive Biosystems 9050 peptide synthesizer using a DIPCDI/HOBt coupling protocol (Table 2, operation 4b). The side-chain hydroxyls of threonine were protected as the *tert*-butyl ethers, and the *N*-terminal pGlu-OH residue was incorporated without further protection. Amino acid analysis of the completed peptide-resin showed the expected ratios: Glu, 1.05; Leu, 0.99; Thr, 1.77; Phe, 0.98; Pro 0.96; Gly, 1.01; Nle, 1.10. Cleavage/deprotection was carried out in two stages by use of TFA−CH_2Cl_2 (1:19), 1 h, 25 °C, followed by filtration and addition of neat TFA to bring the overall filtrate to TFA−CH_2Cl_2 (1:1), 1 h, 25 °C. HPLC analysis directly after cleavage (Figure 5A) showed the presence of desired peptide as well as the two mono-*tert*-butyl and the bis-*tert*-butyl peptide derivatives, whereas HPLC analysis after full deprotection (Figure 5B) indicated the desired peptide as the major peak in >94% purity. The cleavage yield was 89%, and the amino acid composition of the crude cleaved peptide was Glu, 1.08; Leu, 1.03; Thr, 0.89 (destruction upon hydrolysis); Phe, 1.01; Pro 1.00; Gly, 1.05. Cleavage with "dilute" reagent R: TFA−CH_2Cl_2−thioanisole−1,2-ethanedithiol−anisole (5:85:5:3:2) occurred in the same 90% yield, with similar purity after a second step to remove side-chain protecting groups.

Method B. A second synthesis started with Fmoc-2-XAL_4-Nle-MBHA-PS-resin (350 mg, 0.44 mmol/g). The experiment was designed to determine to what extent, if any, the useful results of part A were due to the nature of the support or to the loading. The same protection strategy was employed, but automated synthesis was carried out on a batchwise PerSeptive Biosystems 9600 peptide synthesizer with the BOP/HOBt/NMM protocol (Table 4). The final peptide-resin (502 mg) had the expected weight gain, and its amino acid composition matched theory relative to "internal reference" Nle = 1.08. In several cleavages, both two stage and single step and with or without scavengers, the yields (86−92%), purities (>90%), and amino acid compositions of the desired peptide were indistinguishable from the earlier experiment.

CCK-8 Sulfate [H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2]. Method A. Fmoc-3-XAL_1 (**3a**) was linked to a PEG-PS support (350 mg, 0.21 mmol/g), which comprised PEG:PS in an approximately 1:1 ratio. Couplings of Fmoc-amino acids were performed automatically on a continuous-flow PerSeptive Biosystems 9050 peptide synthesizer using a DIPCDI/HOBt coupling protocol (Table 2, operation 4b). The side-chain carboxyls of aspartate were protected as allyl esters, and other residues were unprotected. After six cycles of amino acid addition, the peptide-resin was transferred to a vessel for manual synthesis, and Fmoc-Tyr(SO_3^-)-OH·$^1/_2Ba^{2+}$ (210 mg, 5 equiv) was introduced using BOP (147 mg, 5 equiv), HOBt (105 mg, 10 equiv), and NMM (156 μL, 15 equiv) in DMF (2 mL), 1 h, 25 °C. Additional cycles removed Fmoc, added Fmoc-Asp(OAl)-OH (DIPCDI/HOBt method, Table 3), and again removed Fmoc. Upon completion of chain assembly, allyl esters were cleaved by use of $Pd(PPh_3)_4$ (50 mg) in $CHCl_3$−HOAc−NMM (20:1:0.5) (3 mL), at 25 °C for 2 h. The resin was next washed (5 mL each) with DMF (3 × 0.5 min), CH_2Cl_2 (3 × 0.5 min), DIEA−CH_2Cl_2 (0.5:99.5) (3 × 2 min), 0.02 M sodium diethyl dithiocarbamate in DMF (3 × 5 min), DMF (3 × 0.5 min), and CH_2Cl_2 (3 × 0.5 min), and dried *in vacuo*.[32] Final cleavage was carried out with TFA−CH_2Cl_2−H_2O (1:18:1), for 15 min, at 25 °C. The overall cleavage yield was 71%, and the desired product represented >95% of the total peptide material, with just traces of the unsulfated peptide (Figure 7A). Amino acid analysis of the crude peptide showed the expected ratios: Asp, 2.02; Tyr, 0.95; Met, 1.94; Gly, 1.05; Phe, 1.04.

Method B. Fmoc-2-XAL_4 (**2b**) was linked to a PEG-PS support (0.15 mg, 0.2 mmol/g), which comprised PEG:PS in an approximately 1:4 ratio. The side-chain carboxyls of aspartate were protected as the *tert*-butyl esters, and other residues were unprotected. Elongation of the chain was carried out essentially as described for method A. A variety of cleavage conditions were examined with the goal to optimize both the cleavage yield and the relative amount of desired sulfate peptide. Optimal cleavage (90% yield) was achieved with TFA−CH_2Cl_2−β-mercaptoethanol−anisole (50:45:3:2), 15 min, 25 °C. The desired product represented ~70% of the total peptide material, with 10% being unsulfated CCK-8 and 20% being CCK-8 sulfate with one or the other of the aspartates retaining *tert*-butyl side-chain protection (Figure 7B). The amino acid composition of the crude peptide was Asp, 2.11; Tyr, 1.03; Met, 1.94; Gly, 0.99; Phe, 0.92.

Oxytocin. Chain assembly was carried out batchwise on a PerSeptive 9600 peptide synthesizer starting with an Fmoc-2-XAL_4-Ala-MBHA-resin (400 mg, 0.08 mmol/g) derived from **2b**. The required Fmoc-amino acids (17.0 equiv) were incorporated by 1 h couplings (except Ile, 90 min) mediated by DIPCDI/HOBt (Table 4, operation 4b). Tyrosine was incorporated with *tert*-butyl side-chain protection, whereas asparagine and glutamine were incorporated as the side-chain unprotected pentafluorophenyl active esters (17.0 equiv for 1 h in the presence of HOBt, *no* DIPCDI). The final peptide-resins (0.06 mmol/g) comprised Asp 0.96, Glu 1.01, Pro 1.20, Gly 0.93, Ile 0.88, Leu 0.98, Tyr 0.89, Cys not determined, Ala 1.05.

Oxytocin (reduced form) was obtained by treatment of the peptide-resin (25 mg) with TFA−CH_2Cl_2−Et_3SiH−H_2O (7:93:0.5:0.5) (2 mL) for 1 h at 25 °C [cleavages were accompanied by formation of significant levels of cyclized peptide, as discussed further in the text]. Oxidations were carried out on peptide-resins (20 mg of peptide-resin) suspended in DMF−anisole (19:1, ∼0.3 mL) containing $Tl(tfa)_3$ [1.1 to 1.3 equiv, final concentration ∼0.5 mM]. After 1 to 2 h at 0 °C, the peptide-resins were washed with DMF and CH_2Cl_2 (4 × 1 min each), then cleaved with TFA−CH_2Cl_2−Et_3SiH−H_2O (7:93:0.5:0.5) (2 mL) for 1 h at 25 °C, and worked up in the standard way. Cleavage yields were typically >90%. Overall yields of cyclized oxytocin were 35−45%. Analytical HPLC evaluations showed good purity (e.g., Figure 9). The crude peptides were verified against a standard from Bachem Biosciences and characterized by FABMS (thioglycerol matrix): calculated monoisotopic mass of $C_{43}H_{66}N_{12}O_{12}S_2$ 1006.4, positive spectrum m/z 1007.4 [MH^+] and 1029.4 [$(M + Na)^+$].

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