Tetrahedron Letters 52 (2011) 7121–7124

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

A convenient procedure for the solid-phase synthesis of hydroxamic acids on PEGA resins

Nitin S. Nandurkar [a], Rico Petersen [a], Katrine Qvortrup [a], Vitaly V. Komnatnyy [a], Kennedy M. Taveras [a], Sebastian T. Le Quement [a], Robin Frauenlob [a], Michael Givskov [b], Thomas E. Nielsen [a,*]

[a] Department of Chemistry, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark
[b] Department of International Health, Immunology and Microbiology, Faculty of Health Sciences, University of Copenhagen, Blegdamsvej 3B, DK-2200 Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 17 September 2011
Revised 7 October 2011
Accepted 18 October 2011
Available online 31 October 2011

Keywords:
Hydroxamic acids
Solid-phase synthesis
Hydroxylamine
HDAC inhibitor
PEGA resin

ABSTRACT

An efficient method for the solid-phase synthesis of hydroxamic acids is described. The method comprises the nucleophilic displacement of esters immobilized on PEGA resins with hydroxylamine/sodium hydroxide in isopropanol. The hydroxyaminolysis protocol is compatible with a broad range of PEGA-supported peptide and peptidomimetic esters. The methodology was found to be compatible with two new strategies for the synthesis of solid-supported lactams and diketopiperazines, respectively, both relying on the high inter- and intramolecular reactivity of cyclic *N*-acyliminium ions with electron-rich aromatics and heteroaromatics, ultimately affording hydroxamic acid derivatives in high purities.

Hydroxamic acids represent an important class of compounds with a wide spectrum of biological properties, such as antibacterial, antifungal and anticancer (Fig. 1).[1,2] The ability of hydroxamates to chelate metal ions, such as Fe^{3+} and Zn^{2+}, has been widely explored in biology and medicine, as exemplified by hydroxamic acid based siderophores produced by microorganisms for the harvesting of iron from iron-deficient environments. The siderophore desferrioxamine B (Desferal, **1**) is used medically to treat iron poisoning, which can arise following blood transfusion to patients with genetic blood diseases.[3]

A large number of recent studies have dealt with hydroxamic acids as potent inhibitors of Zn^{2+}-containing enzymes, such as matrix metallo-proteinases (MMPs), and histone deacetylases (HDACs) in particular. Although hydroxamic acids have been widely explored in the pharmaceutical industry for decades, the number of clinical failures associated with this compound class is substantial (e.g., for **3** and **4**). However, the launch of the HDAC inhibitor, vorinostat (SAHA, suberoyl anilide hydroxamic acid, **2**) in 2006 by Merck for the treatment of cutaneous T-cell lymphoma,[4] has drawn renewed attention to the chemistry and biomedical properties of hydroxamic acids.

Given the growing number of potent hydroxamic acids identified in drug and probe discovery efforts,[5] methods for the parallel and combinatorial synthesis of hydroxamic acids have been widely explored. There have been several reports describing the solid-phase synthesis of hydroxamic acids.[6] For example, hydroxylamine derivatives, being either *N*-tethered to MBHA,[7] and Tentagel resins,[8] or *O*-tethered to Wang,[9] Sasrin,[10] and trityl resins,[11] have served as the starting point for the synthesis of a range of hydroxamic acids. Another approach has been a stepwise method, where esters were cleaved from the resin to give the corresponding carboxylic acids that were subsequently reattached to a hydroxylamine resin using

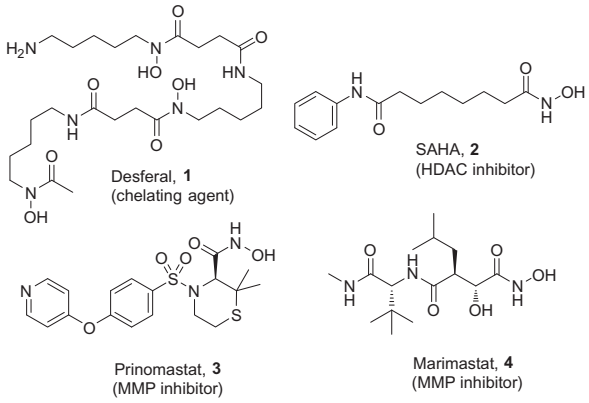


Figure 1. Clinically tested hydroxamic acids.

* Corresponding author. Tel.: +45 4525 2134; fax: +45 4593 3968.
E-mail address: ten@kemi.dtu.dk (T.E. Nielsen).

0040-4039/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2011.10.103

a peptide coupling agent, followed by cleavage to provide the hydroxamic acids.[12]

The solid-phase synthesis of hydroxamic acids via direct hydroxyaminolysis of an ester-linked substrate has also been reported. These methods generally require treatment of the esterified resin with excess amounts of NH_2OH for prolonged periods of time to generate effectively the corresponding hydroxamic acids.[13] In a few reports, activated resins have been employed to facilitate a more rapid cleavage.[14] In one highly interesting report, the hydroxyaminolysis of an ester-linked substrate using aqueous hydroxylamine and KCN as the catalyst was also reported.[15] As part of ongoing efforts to synthesize hydroxamic acids, we herein communicate our results on the hydroxyaminolysis of ester-linked substrates on PEGA resins, thus providing a convenient procedure for the release of hydroxamic acids from the solid support.

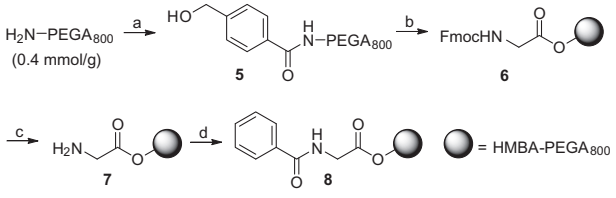
Initially, a solid-supported substrate (**8**, Scheme 1), to be used for rapid optimization of the reaction conditions necessary for hydroxamic acid release, was constructed.

Starting with amino-functionalized $PEGA_{800}$ resin (0.4 mmol/g),[16] the HMBA (hydroxymethylbenzoic acid) linker was attached using a *N,N,N′,N′*-tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate (TBTU) mediated amide coupling procedure.[17] The HMBA linker was then esterified with Fmoc-Gly-OH using the 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT) protocol.[18] Fmoc deprotection with 20% piperidine in DMF and final TBTU-coupling of benzoic acid then yielded solid-supported substrate **8**. The purity of **8** (>95%) was tested via release from the HMBA linker, as the corresponding carboxylic acid, with aqueous NaOH (0.1 M).[19]

With solid-supported ester-linked substrate **8** in hand several cleavage reaction conditions were tested. Using 50% aqueous NH_2OH (13 equiv), a range of protic solvents and basic additives were first examined (see Table 1 for selected results).

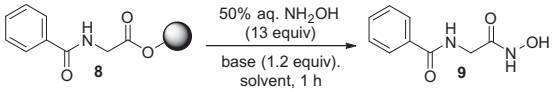
The results showed how the combination of NH_2OH with NaOH in *i*-PrOH or MeOH was extremely efficient for the formation of the desired hydroxamic acid **9** in excellent purity (>95%). A range of bases (KOH, NaOH, KO*t*-Bu) and solvents (toluene, 1,4-dioxane, DMF, and THF) was examined (results not shown), but none proved nearly as efficient as those noted in Table 1. Critical for a successful hydroxylaminolysis reaction is good solubility of the base in the solvent, which must still be able to swell effectively the resin, while not transforming the formed hydroxamic acid further. Interestingly, when the cleavage was performed in the absence of base, hydroxamic acid **9** could be obtained in good purity (84%). A rapid examination of the reagent stoichiometries showed the importance of maintaining a low amount of base, as decomposition of the hydroxamic acid **9** was observed under strongly basic conditions.

Having developed an efficient protocol for the release of hydroxamic acid **9** from the solid support, we next investigated the scope of the methodology for a range of solid-supported esters (Table 2).[19] All the ester-linked substrates were synthesized according to the synthetic strategy presented in Scheme 1.



Scheme 1. Solid-phase synthesis of ester-linked substrate **8**. Reagents and conditions: (a) HMBA, TBTU, NEM, DMF; (b) FmocGlyOH, MSNT, MeIm, CH_2Cl_2; (c) 20% piperidine (DMF), (d) $PhCO_2H$, TBTU, *N*-ethylmorpholine (NEM), DMF.

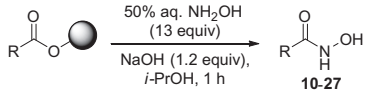
Table 1
Optimization study for the conversion of resin-bound ester **8** into the corresponding hydroxamic acid **9**



Entry	Base	Solvent	Purity (%)[a]
1	None	MeOH	84
2	NaOH	*t*-BuOH	88
3	NaOH	*i*-PrOH	>95
4	NaOH	MeOH	>95
5	KOH	MeOH	92

[a] Determined by RP-HPLC/MS of the crude products (254 nm).

Table 2
Conversion of ester-linked substrates into the corresponding hydroxamic acids **10**–**27** via hydroxyaminolysis[a]



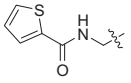
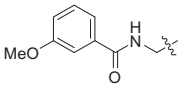
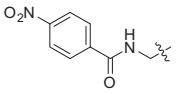
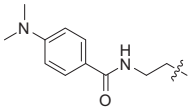
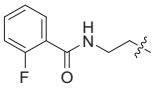
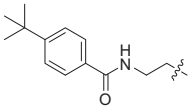
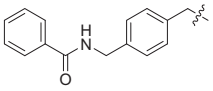
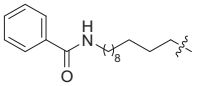
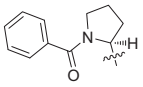
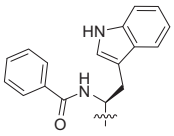
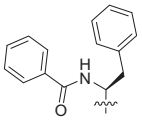
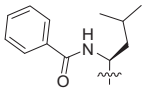
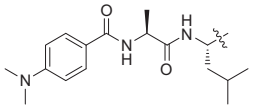
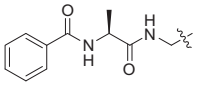
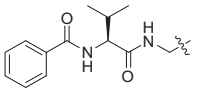
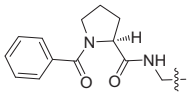
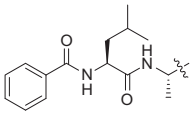
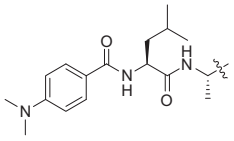
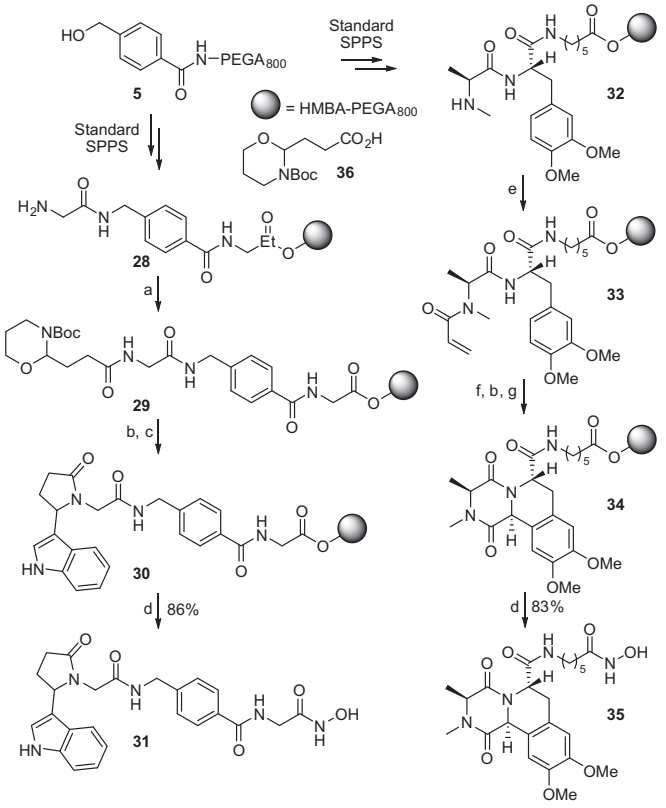
Entry	R	Product	Purity (%)[b]
1		**10**	92
2		**11**	91
3		**12**	69
4		**13**	>95
5		**14**	82
6		**15**	>95
7		**16**	84
8		**17**	86
9		**18**	55
10		**19**	74
11		**20**	94

Table 2 (*continued*)

Entry	R	Product	Purity (%)[b]
12		**21**	89
13		**22**	87
14		**23**	93
15		**24**	91
16		**25**	>95
17		**26**	>95
18		**27**	>95

[a] A reaction time of 1 h gave the crude product in >80% yield from 50 mg of resin.
[b] As determined by RP-HPLC/MS of the crude products (254 nm).



Scheme 2. Solid-phase synthesis of compounds **31** and **35**. Reagents and conditions: (a) **36**, TBTU, NEM, DMF; (b) 10% TFA (aq); (c) indole, 50% TFA (CH_2Cl_2); (d) 50% NH_2OH, NaOH, *i*-PrOH; (e) $CH_2CHCOCl$, CH_2Cl_2; (f) OsO_4, $NaIO_4$, DBU, H_2O:THF (1:1); (g) 50% TFA (CH_2Cl_2).

A series of unhindered esters incorporating substituted aromatic amide moieties was examined (entries 1–8). Gratifyingly, the protocol proved well compatible with these substrates, generally providing the desired hydroxamic acids in reasonable to excellent purities (69→95%). In further experiments, the compatibility with sterically more congested substrates was also demonstrated (entries 10–18), revealing a synthetically useful protocol giving hydroxamic acids in excellent purities (74→95%). Some limitation was observed for the more hindered proline derivative **18** (entry 9), that could only be formed in moderate purity (55%).

Efforts in our laboratories have dealt with the use of solid-supported *N*-acyliminium intermediates,[20] conveniently formed by intramolecular aldehyde-amide condensation reactions, for the synthesis of a wide range of constrained peptidomimetic scaffolds.[21] In this context, we have been particularly interested in the solid-phase synthesis of tetrahydro-β-carbolines and tetrahydroisoquinolines (THIQs), which are known to exhibit a plethora of biological activities.[22] To advance the scope of this methodology, we sought to demonstrate that the protocol shown in Table 2 could also be applied in this context. Previous efforts have focused on intramolecular reactions of cyclic *N*-acyliminium intermediates. Below, we present two new variants thereof, one useful for the synthesis of substituted lactams, the other applicable to the synthesis of polycyclic diketopiperazines. In this context, compounds **28** and **32** (Scheme 2) were constructed on a solid support using standard solid-phase synthesis protocols. Compound **28** was acylated with masked aldehyde building block **36**,[23] prior to reaction with indole as an external nucleophile under acidic reaction conditions.[19] The desired lactam hydroxamic acid **31** was subsequently released in 86% purity from the solid support. Similarly, acryloylated peptide **33** was subjected to OsO_4/$NaIO_4$-mediated oxidative cleavage,[24] followed by treatment with 50% TFA (CH_2Cl_2).[19] The resulting diketopiperazine hydroxamic acid **35** was finally isolated in high purity (83%). In both cases, only minor amounts of the corresponding carboxylic acids were observed (<10%).

In summary, an efficient protocol for the solid-phase synthesis of hydroxamic acids has been developed. The protocol relies on readily available HMBA esters, which are easily accessible on PEGA supports. Hydroxamic acids were then efficiently formed following hydroxyaminolysis with NH_2OH/NaOH in isopropanol. The methodology is useful for the synthesis of peptidic and peptidomimetic hydroxamic acids in good to excellent purities. In addition, two new *N*-acyliminium reactions for the synthesis of substituted lactams and diketopiperazines, respectively, were also developed. Both heterocyclic scaffolds were synthesized effectively, embedded in highly pure products released from the solid support, thus paving the way for the synthesis of larger and structurally more complex libraries of hydroxamic acids. Biological evaluation of the synthesized hydroxamic acids as HDAC inhibitors is ongoing, and the results will be reported in due course.

Acknowledgments

The Hans Christian Oersted Postdoc Program, Technical University of Denmark (DTU), Carlsberg Foundation, Lundbeck Foundation, Danish Councils for Strategic and Independent Research, and Holm Foundation are gratefully acknowledged for the financial support.

Supplementary data

Supplementary data (experimental synthetic procedures and data for selected compounds) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.10.103.

References and notes

1. Rappoport, Z.; Liebman, J. F. In Rappoport, Z., Ed.; The Chemistry of Hydroxylamines, Oximes and Hydroxamic Acids; Wiley-Interscience: Chichester, 2009; Vols. 1–2,.
2. (a) Miller, M. J. *Acc. Chem. Res.* **1986**, *19*, 49–56; (b) Miller, M. J. *Chem. Rev.* **1989**, *89*, 1563–1579.
3. (a) Bickel, H.; Bosshardt, R.; Gäumann, E.; Reusser, P.; Vischer, E.; Voser, W.; Wettstein, A.; Zähner, H. *Helv. Chim. Acta* **1960**, *43*, 2118–2128; (b) Fischer, E. A.; McLachlan, D. R.; Kruck, T. P. A.; Mustard, R. A. *Pharmacology* **1990**, *41*, 263–271; (c) Porter, J. B.; Rafique, R.; Srichairatanakool, S.; Davis, B. A.; Shah, F. T.; Hair, T.; Evans, P. *Ann. N.Y. Acad. Sci.* **2005**, *1054*, 155–168.
4. (a) Richon, V. M.; Webb, Y.; Merger, R.; Sheppard, T.; Jursic, B.; Ngo, L.; Civoli, F.; Breslow, R.; Rifkind, R. A.; Marks, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5705–5708; (b) Mai, A.; Esposito, M.; Sbardella, G.; Massa, S. *Org. Prep. Proced. Int.* **2001**, *33*, 391–394.
5. (a) Saban, N.; Bujak, M. *Cancer Chemother. Pharmacol.* **2009**, *64*, 213–221; (b) Paris, M.; Porcelloni, M.; Binsachi, M.; Fattori, D. *J. Med. Chem.* **2008**, *51*, 1505–1529.
6. (a) Krchnák, V. *Mini-Rev. Med. Chem.* **2006**, *6*, 27–36; (b) Alsina, J.; Albericio, F. *Biopolymers* **2003**, *71*, 454–477.
7. Chen, J. J.; Spatola, A. F. *Tetrahedron Lett.* **1997**, *38*, 1511–1514.
8. Ngu, K.; Patel, D. V. *J. Org. Chem.* **1997**, *62*, 7088–7089.
9. (a) Floyd, C. D.; Lewis, C. N.; Patel, S. R.; Whittaker, M. *Tetrahedron Lett.* **1996**, *37*, 8045–8048; (b) Richter, L. S.; Dessai, M. C. *Tetrahedron Lett.* **1997**, *38*, 321–322.
10. (a) Gordeev, M. F.; Hui, H. C.; Gordon, E. M.; Patel, D. V. *Tetrahedron Lett.* **1997**, *38*, 1729–1732; (b) Barlaam, B.; Koza, P.; Berriot, J. *Tetrahedron* **1999**, *55*, 7221–7232.
11. (a) Mellor, S. L.; McGuire, C.; Chan, W. C. *Tetrahedron Lett.* **1997**, *38*, 3311–3314; (b) Bauer, U.; Ho, W. B.; Koskinen, A. M. P. *Tetrahedron Lett.* **1997**, *38*, 7233–7236.
12. Salvino, J. M.; Mathew, R.; Kiesow, T.; Narensingh, R.; Mason, H. J.; Dodd, A.; Groneberg, R.; Burns, C. J.; McGeehan, G.; Kline, J.; Orton, E.; Tamg, S.-H.; Morrisette, M.; Labaudininiere, R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1637–1640.
13. (a) Dankwardt, S. M. *Synlett* **1998**, 761; (b) Dankwardt, S. M.; Billedeau, R. J.; Lawley, L. K.; Abbot, S. C.; Martin, R. L.; Chan, C. S.; Van Wart, H. E.; Walker, K. E. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2513–2516.
14. (a) Zhang, W.; Zhang, L.; Li, X.; Weigel, J. A.; Hall, S. E.; Mayer, J. P. *J. Comb. Chem.* **2001**, *3*, 151–153; (b) Thouin, E.; Lubell, W. D. *Tetrahedron Lett.* **2000**, *41*, 457–460.
15. Ho, C. Y.; Strobel, E.; Ralbovsky, J.; Galemmo, R. A., Jr. *J. Org. Chem.* **2005**, *70*, 4873–4875.
16. Meldal, M. *Tetrahedron Lett.* **1992**, *33*, 3077–3080.
17. Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927–1930.
18. Blankemeyer-Menge, B.; Nimtz, M.; Frank, R. *Tetrahedron Lett.* **1990**, *31*, 1701–1704.
19. *General solid-phase peptide synthesis procedures:* Attachment of HMBA linker to amino-functionalized $PEGA_{800}$ resin was carried out by pre-mixing HMBA (3.0 equiv), NEM (4.0 equiv) and TBTU (2.88 equiv) in DMF for 5 min. The volume of DMF was kept to a minimum amount sufficient for full coverage and swelling of the beads. The resulting solution was added to the resin, and allowed to react for 2 h. After removal of the reaction mixture by suction, the remaining resin was washed with DMF (×6), CH_2Cl_2 (×6), and then dried under vacuum. Coupling of the first amino acid to the HMBA-derivatized resin was accomplished by treating the freshly lyophilized resin with a mixture of the Fmoc-protected amino acid (3.0 equiv), MeIm (5.0 equiv) and MSNT (3.0 equiv) in CH_2Cl_2. The MSNT-mediated coupling was repeated once. Removal of the Fmoc group was carried out by treating the resin with 20% piperidine in DMF, first for 2 min, followed by washing with DMF (×2), and then for 18 min. The deprotected resin was then washed with DMF (×6), CH_2Cl_2 (×6) and then DMF (×6) again. Subsequent attachment of amino acids and/or capping units was accomplished using appropriate cycles of TBTU-mediated coupling and Fmoc deprotection reactions as described above.
General procedure for the synthesis of hydroxamic acids by hydroxyaminolysis of ester-linked substrates: NaOH (1.2 equiv) was weighed and dissolved in a minimum amount of *i*-PrOH. A solution of 50% NH_2OH (aq) (13 equiv) was then added. The solution was transferred to the resin and the swollen resin was gently stirred with a spatula. After cleavage for 15 h, the cleavage solution was drained from the resin and the resin rinsed with *i*-PrOH (×2). The combined solutions were neutralized with 0.1 M HCl (aq), the solvents evaporated, and the resulting residue lyophilized overnight.
*Procedure for the synthesis of substrate **30** from **29**:* Solid-supported masked aldehyde substrate **29** was treated with 10% TFA (aq) for 1 h. The resin was filtered, washed with H_2O (×6), DMF (×6), MeOH (×6) and CH_2Cl_2 (×6), and lyophilized for 20 h. The resin was then treated with indole (3 equiv) in 50% TFA (CH_2Cl_2). The reaction was allowed to proceed for 5 min. After removal of the reaction mixture by suction, the remaining resin was washed with H_2O (×6), 20% piperidine in DMF (×2), H_2O (×6), DMF (×6), MeOH (×6) and CH_2Cl_2 (×6), dried under vacuum and lyophilized.
*Procedure for the synthesis of substrate **34** from **32**:* Acryloyl chloride (10 equiv) was dissolved in dry CH_2Cl_2 and transferred to solid-supported peptide **32**. The swollen resin was stirred gently with a spatula and allowed to react for 1 h. The resin was filtered and washed with H_2O (×6), DMF (×6) and CH_2Cl_2 (×6), then dried using suction for 30 min. The resin was transferred to a capped reaction vial. The oxidative cleavage was carried out by addition of DBU (5 equiv) and $NaIO_4$ (10 equiv) to the resin pre-swollen in H_2O:THF (1:1). After 10 min of shaking, a solution of OsO_4 in *t*-BuOH (2.5% w/w, 0.05 equiv) was added and the mixture was left under shaking for 20 h. The mixture was transferred to a syringe (5 ml) equipped with a polypropylene filter. After removal of the reaction mixture by suction, the resin was washed with H_2O (×6), 10% TFA (aq) (×4), H_2O (×6), DMF (×6) and CH_2Cl_2 (×6) and dried under vacuum. The resin was then subjected to treatment with 50% TFA (CH_2Cl_2), stirred gently with a spatula and allowed to react, in a sealed syringe, for 25 h. After removal of the reaction mixture by suction, the remaining resin was washed with H_2O (×6), 20% piperidine in DMF (×2), H_2O (×6), DMF (×6), MeOH (×6) and CH_2Cl_2 (×6), dried under vacuum and lyophilized.
*Compound **35***: ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.31 (br s, 1H), 8.14 (m, 1H), 6.94 (s, 1H), 6.73 (s, 1H), 5.10 (s, 1H), 4.56 (dd, J = 8.5, 7.5 Hz, 1H), 4.09 (q, J = 6.8 Hz, 1H), 3.75 (s, 3H), 3.68 (s, 3H), 3.14 (dd, J = 15.5, 7.2 Hz, 1H), 3.05 (m, 2H), 3.01 (s, 3H), 2.90 (dd, J = 15.0, 9.1 Hz, 1H), 1.92 (m, 2H), 1.46 (m, 2H), 1.38 (m, 2H), 1.19 (m, 2H), 1.11 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.4, 168.9, 167.0, 162.8, 148.4, 147.0, 126.7, 126.5, 111.7, 107.4, 56.1, 55.3, 55.2, 53.0, 38.1, 31.8, 30.9, 29.1. 28.4, 25.4, 24.5, 17.7; MS (ESI) calcd for $C_{23}H_{33}N_4O_7$ $[M+H]^+$ 477.2 found 477.3. Isolated yield after prep. RP-HPLC: 15%.
20. For a review on the use of *N*-acyliminium intermediates on solid-phase, see: Le Quement, S. T.; Petersen, R.; Meldal, M.; Nielsen, T. E. *Biopolymers* **2010**, *94*, 242–256.
21. For examples, see: (a) Le Quement, S. T.; Nielsen, T. E.; Meldal, M. *J. Comb. Chem.* **2008**, *10*, 447–455; (b) Le Quement, S. T.; Nielsen, T. E.; Meldal, M. *J. Comb. Chem.* **2007**, *9*, 1060–1072; (c) Nielsen, T. E.; Le Quement, S.; Meldal, M. *Org. Lett.* **2005**, *7*, 3601–3604; (d) Nielsen, T. E.; Meldal, M. *J. Comb. Chem.* **2005**, *7*, 599–610; (e) Nielsen, T. E.; Meldal, M. *J. Org. Chem.* **2004**, *69*, 3765–3773.
22. Zarranz De Ysern, M. E.; Ordoñez, L. A. *Prog. Neuro-Psychopharmacol.* **1981**, *5*, 343–355.
23. (a) Groth, T.; Meldal, M. *J. Comb. Chem.* **2001**, *3*, 34–44; (b) Groth, T.; Meldal, M. *J. Comb. Chem.* **2001**, *3*, 45–63.
24. Nielsen, T. E.; Meldal, M. *Org. Lett.* **2005**, *7*, 2695–2698.