Proceedings of the 31st European Peptide Symposium
Michal Lebl, Morten Meldal, Knud J. Jensen, Thomas Hoeg-Jensen (Editors)
European Peptide Society, 2010

DTT Reacts with TFA to Form a Novel Bicyclic Dithioorthoester

Jan Pawlas[1], Stefan Hansen[1], Anne H. Sørensen[1], Gunnar Stærkær[1], Anette Møller[1], Neil Thompson[1], Thomas Pagano[2], Fangming Kong[2], Steve Koza[2], Mark Pozzo[3], Jari Finneman[3], and Patricia Droege[3]

[1]PolyPeptide Laboratories A/S, Herredsvejen 2, 3400, Hillerød, Denmark; [2]Pfizer Global Biologics, One Burtt Rd., Andover, MA, 01810, U.S.A.; [3]Pfizer Global Biologics, 700 Chesterfield Parkway West, St Louis, MO, 63017, U.S.A.

Introduction

Dithiothreitol (DTT, Cleland's reagent [1]) is a cheap, non-odorous reducing agent [2], which is produced by a safe and environmentally friendly process [3]. In fact, DTT was recently used as a scavenger during large scale preparation of the HIV fusion inhibitor Fuzeon [4]. We now report that DTT reacts with trifluoroacetic acid (TFA) to form a bicyclic dithioorthoester which can be difficult to detect by HPLC.

Results and Discussion

During a large scale production of a 37-mer peptide, we found that solutions of the peptide contained small amounts of an impurity, which were not easily detected by HPLC. At low peptide concentrations during purification, this impurity was virtually undetectable by HPLC. However, this material could be detected by ^{1}H NMR in highly concentrated samples of the peptide obtained after lyophilisation. Furthermore, we found that performing HPLC analyses with the UV detector set at 210 nm, this compound could be detected much easier than for example at 220 nm (see Figure 1). We set out to identify this compound and after some experimentation we were able to isolate a small quantity of this material from its mixture with the peptide. The bicyclic dithioorthoester **1** is fully consistent with the NMR and MS data for the isolated material, and we propose that this compound is formed via the dithianylium salt **2** (Figure 2). In fact, it is known that dithiols react with trifluoroacetic anhydride in the presence of strong acids to give dithianylium salts, which can be converted to α-trifluoromethyl substituted dithioorthoesters upon treatment with alcoholates [5].

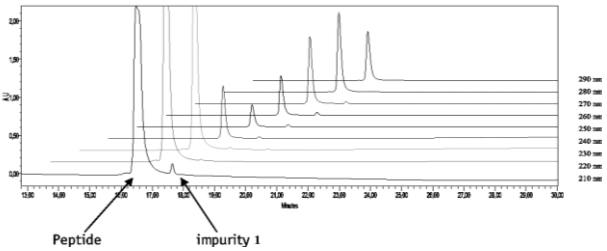

*Fig. 1. Overlay of chromatograms of a 37-mer peptide (ca 20 g/l) containing **1**.*

The peptide in which we detected dithioorthoester **1** was prepared by a standard Fmoc SPPS-cleavage-RP HPLC-lyophilisation process. Thus, the peptide resin was exposed to a DTT containing TFA cocktail and it is therefore conceivable that **1** was formed during the cleavage step of the process. In order to establish the precise conditions required for the formation of **1**, we allowed DTT to react with TFA in the presence of various additives common in cleavage cocktails. In all these experiments, DTT was fully consumed upon standing in TFA, and regardless of the presence of other reactants, **1** was formed as a major component of the reaction mixture. In fact, simply mixing DTT with TFA in the presence of triisopropylsilane (TIS) afforded **1** essentially quantitatively [6]. Finally, it is worth noting that DTT was completely stable when TFA (pK_a 0.30) was replaced by acetic acid (pK_a 4.76).

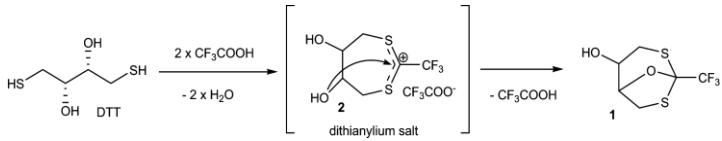

*Fig. 2. Proposed mechanism of formation of **1** via dithianylium salt **2**.*

We next sought practical conditions to remove **1** from the 37-mer peptide we had at hand. Extraction of **1** with toluene from an aqueous solution of the peptide did remove this non-peptide impurity completely. Nevertheless, on large scale it can be more convenient to purify peptides by chromatography and we set out to develop a process to remove **1** using RP HPLC. Towards this end, we examined several polymers as stationary phases and a range of mobile phases for their efficiency of removal of **1**.

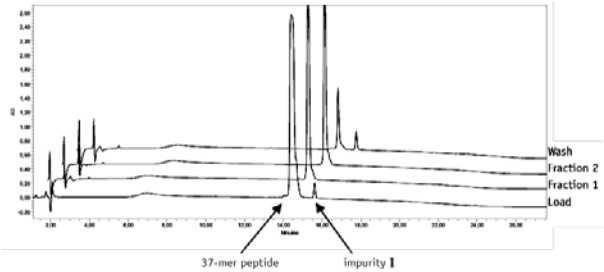

*Fig. 3. Removal of impurity **1** from a 37-mer peptide using RP HPLC [7].*

Interestingly, in all the purifications using silica C18 stationary phases, regardless of pH or the presence of various organic modifiers, the compound **1** (MW 232) coeluted with the peptide (MW > 4000). On the contrary, using polystyrene divinylbenzene (PS/DVB) based stationary phases, we were able to separate **1** from the peptide with every mobile phase system that we examined. An example of a purification using a PS/DVB resin is shown in Figure 3. Note that the main (peptide) peak contains some peptide related impurities as the test purification shown here was carried out using a batch of ca 90% pure 37-mer peptide. The purpose of this purification was solely to remove **1** and not to remove peptide related impurities.

In conclusion, we report that DTT in the presence of TFA forms a novel bicyclic dithioorthoester **1**. This compound can be difficult to detect by HPLC, especially with the UV detector set at 220 nm. The structural assignment that we propose is in full agreement with the spectral data and the formation of **1** can be explained by the mechanism shown in Figure 2. Although the choice of stationary phase was crucial for the removal of **1** from a 37-mer peptide using RP HPLC, a different set of conditions may be required for removal of **1** from another peptide.

Acknowledgments
We thank Ms. Sylvia Ritz for editorial assistance.

References

1. Cleland, W.W. *Biochemistry* **3**, 480 (1964).
2. Houghten, R.A., Li, C.H. *Anal. Biochem*. **98**, 36 (1979).
3. Bruckdorfer, T., Marder, O., Albericio, F. *Curr. Pharm. Biotechnol*. **5**, 29 (2004).
4. Zhang, H., Schneider, S.E., Bray, B.L., Friedrich, P.E., Tvermoes, N.A., Mader, C.J., Whight, S.H., Niemi, T.E., Silinski, P., Picking, T., Warren, M., Wring, S.A. *Org. Process Res. Dev*. **12,** 101 (2008).
5. Sevenard, D.V., Kirsch, P., Lork, E., Röschenthaler, G.-V. *Tetrahedron Lett*. **44**, 5995 (2003).
6. 1-(Trifluoromethyl)-8-oxa-2,7-dithia-bicyclo[3.2.1]octan-4-ol (1). At room temperature (rt) with stirring, neat TFA (30.0 ml, 403.8 mmol, 20.8 equiv) was added to DTT (3.0 g, 19.4 mmol, 1.0 equiv) and TIS (3.0 ml, 24.6 mmol, 1.3 equiv). The resulting solution was stirred at rt for 48 hours, after which DTT was fully consumed (HPLC) and the reaction was poured into 1.0 M aqueous HCl (200 ml). The suspension thus formed was extracted with ether (200 ml), the organic phase was dried ($MgSO_4$) and volatiles were removed in vacuo to afford the title compound (4.2 g, 93%) as an off-white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.85 (d, *J*=5.0 Hz, 1H) 5.00 (bd, *J*=5.1 Hz, 1H), 3.86 (m, 1H), 3.61 (d, *J*=10.2 Hz, 1H), 3.33 (dd, *J*=10.2 Hz, 6.7Hz, 1H), 3.23 (dd, *J*=12.9 Hz, 10.7 Hz, 1H), 3.15 (dd, *J*=12.9 Hz, 5.1 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 122.8 (q), 99.1 (q), 84.5, 63.2, 32.8, 29.0. ^{19}F NMR (376 MHz, DMSO-d6) δ 73.7 HRMS *m/z* measured: 231.98351, calcd: 231.98395.
7. Stationary phase: Amberchrom XT20; mobile phase: A buffer - 1 mM HCl in water, B buffer – CH_3CN, recorded at 210 nm. Fractions 1 and 2 were obtained by eluting with 20-30% B, impurity 1 was eluted with >40% B (wash).