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Oxidation of threonine residues with IBX reagents

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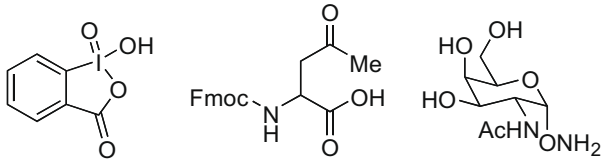
ABSTRACT

The treatment of protected Thr peptides with excess polymer-bound IBX effects sequential oxidation of the Thr secondary alcohol to the ketone, followed by hydroxylation of the alpha carbon of the Thr residues with loss of stereochemistry at this position.

The hypervalent iodine agent *o*-iodoxybenzoic acid **1** (IBX) is a mild oxidising agent that has been applied widely in organic chemistry[1] since it was reported to oxidise alcohols in 1994.[2] The scope of the reagent has been significantly extended with the development of both water-soluble derivatives[3] and several polymer-bound IBX reagents.[4,5] The reaction mechanism is highly dependent on the specific IBX reagent employed and solvent effects are important in the selectivity of the oxidations and the type of oxidation products that are formed.[1,3,6,7]

While IBX and its derivatives have been used to oxidise a wide variety of substrates including alcohols, silyl enol ethers, amines and amides,[1–7] there are limited applications of these reagents in peptide chemistry,[5] and there are no examples of the use of these reagents to oxidise the side chains in Ser or Thr in either amino acids or peptides. The incorporation of an aldehyde or ketone into a peptide chain is an important reaction as it allows selective functionalisation at a single site by the formation of Schiff base derivatives. Bertozzi and co-workers have elegantly demonstrated this approach with site-specific introduction of a carbonyl group into peptides and proteins via solid-phase peptide synthesis or enzymatic oxidation of glycosidated residues.[8] For example, the unnatural amino acid **2**[9] was introduced into peptides via solid-phase synthesis followed by reaction with the amino-oxy-sugar derivative of GalNAc **3**[8,10] to give stable oxime derivatives that were biologically active and hence acted as excellent mimics of natural glycosidated peptides.[8]



1 **2** **3**

As part of our studies on the design and synthesis of mimics of naturally occurring fish antifreeze glycoproteins (AFGPs) (Fig. 1a), we were interested in the application of IBX reagents to the oxidation of the side-chain of Thr residues in small peptides and building blocks suitable for the assembly of polypeptides via chemical ligation.[11] The direct introduction of the ketone functional group would allow easy addition of the GalNAc sugar via reaction with the amino-oxo sugar **3** to give AFGP mimics (Fig. 1b). The design of AFGP mimics is only possible now due to recent structure–activity studies of AFGPs that have identified the critical requirement for the Thr-γ-methyl group and the presence of the GalNAc sugar as an α-glycoside.[12] In addition, oxidation of Thr side chains would allow direct entry to a range of derivatives of peptides by the addition of different nucleophiles, which is of interest for both the introduction of labels into peptides and proteins as well as in combinatorial chemistry for the introduction of diversity into peptide libraries.

In this Letter we report the application of polymer-bound IBX (pIBX) to the oxidation of protected Thr and Thr side chains in polypeptides. While the desired side chain oxidation occurred, unexpected oxidation at the Thr α-C was also observed to provide a new class of peptide derivatives.

pIBX is an attractive reagent for applications with peptides as removal of the resin following the reaction should allow access to pure product requiring minimal purification.[4] While oxidation

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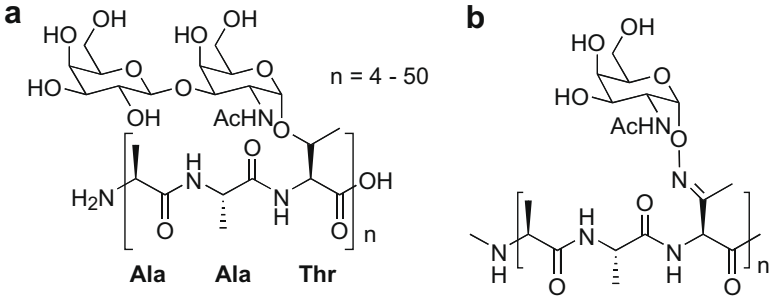


Figure 1. (a) Structure of naturally occurring AFGPs; (b) proposed AFGP mimic.

of the side chain of Thr has been reported using a variety of conditions[13] including Dess–Martin periodinane, CrO_3, PCC and dimethyl dioxirane, these reagents have specific solvent requirements that are generally not suitable for peptides or for the very hydrophobic peptide backbone present in our target AFGP mimics (Fig. 1b).

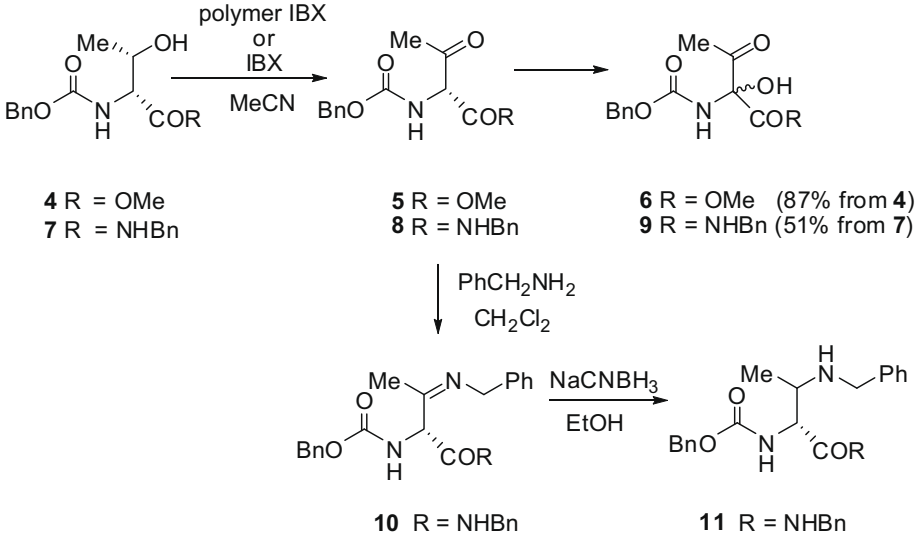
The optimal reaction conditions for oxidation of the secondary alcohol side chain in Thr to the corresponding ketone with pIBX were determined using a simple NMR assay with Z-Thr as the substrate and MeCN-d_3 as solvent. The progress of the reaction was monitored by the disappearance of the Me doublet in Z-Thr at 1.17 ppm and by the appearance of the ketone Me singlet at 2.37 ppm. In the presence of 2 equiv of pIBX at room temperature, no oxidation occurred after 24 h. Increased equivalents of pIBX (4 equiv) resulted in formation of the ketone but significant amounts of unreacted alcohol remained (3:1). Quantitative conversion of Z-Thr to the ketone required 10 equiv of pIBX and heating at 45 °C for 2.5 days. The reaction tolerated the presence of cosolvents DME or DMF (25%), but required significantly longer reaction times of up to 3–4 days. However, the addition of water (30–50%) resulted in no reaction due to inhibition of polymer swelling.[5]

The optimised conditions (10 equiv pIBX, MeCN, 45 °C, 2 d) were applied to the methyl ester **4** and amide **7** protected Thr derivatives and resulted in complete oxidation of the Thr side chain in both cases (Scheme 1). However, the elevated temperature resulted in some minor leaching of the polymer resin into the solution, as evidenced by the appearance of aliphatic multiplets in the NMR spectrum of the crude products, requiring purification by chromatography.

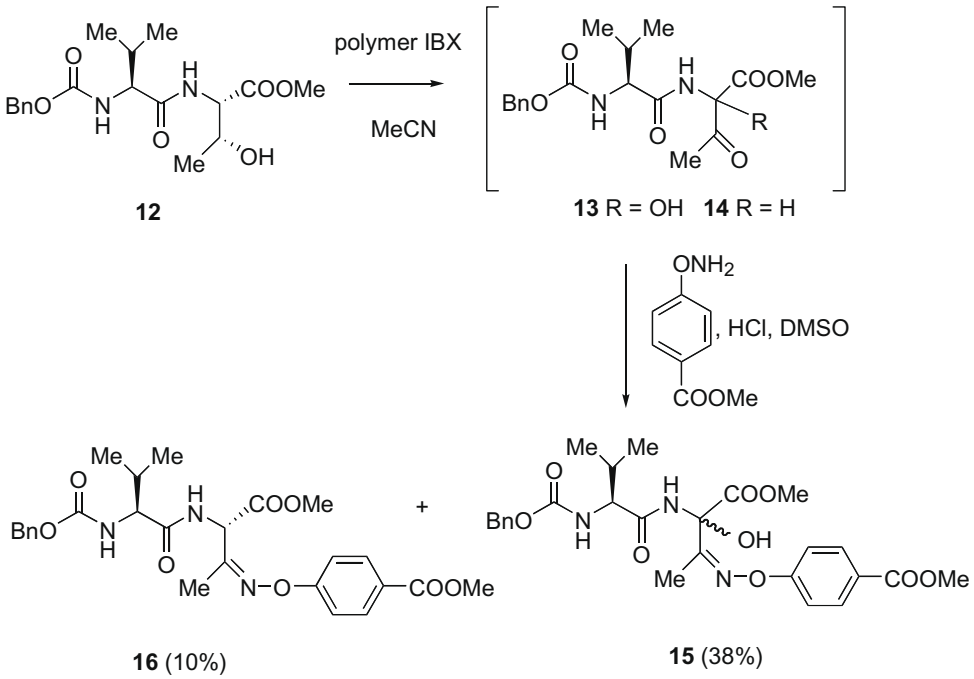
In the case of the methyl ester **4**, analysis of the product showed none of the desired material **5**, but instead the oxidised product **6** was formed (Scheme 1). ^{1}H NMR analysis showed the absence of the characteristic Thr H-α proton expected in **5**. In addition, a signal that integrated for 1H and exchanged with D_2O was present at 5.31 ppm. Keto-enol tautomerism has been reported in a number of related peptides and amino acid derivatives and could explain these properties.[14] However, the ^{13}C NMR spectrum of **6** showed clear evidence for the new ketone carbonyl at 197.9 ppm and no evidence for a possible enol tautomer of **5**. Changing the solvent from MeCN to MeOH did not change the appearance of the spectrum consistent with the absence of tautomerism. The high-resolution mass spectrum of the product identified that an additional oxygen atom was present, and hence all data were consistent with the formation of the α-substituted product **6**.[15] This assignment was confirmed by DEPT and HSQC spectra.

On one occasion where the reaction had not gone to completion, analysis of the Me signals in the crude product was consistent with formation of the desired ketone **5** as a very minor product (<10%) along with **6**. Both compounds had very similar R_f values and the low yield of **5** made isolation difficult.[16]

Similar oxidation products were obtained with the amide derivative **7**. Thus, treatment of the amide **7** with 10 equiv of pIBX in MeCN resulted in formation of the corresponding α-hydroxy-substituted ketone **9**. However, compared to the reaction with ester **4**, a significantly higher amount of the desired ketone **8** was formed (25%) and while both **8** and **9** had similar polarities, both products were isolated and fully characterised.[17] The pure ketone



Scheme 1.



Scheme 2.

8 was converted to the imine **10** (δ $CH_3C{=}N$ 2.16 ppm) which was reduced to the amine **11** with sodium cyanoborohydride.

The results of treatment of both **4** and **7** with pIBX were consistent with initial oxidation of the Thr side chain, followed by oxidation at the C-α position to give **6** and **9**, respectively, due to the excess pIBX used in the reaction. The two stepwise oxidation reactions (Scheme 1) were confirmed by monitoring the reaction by ^{1}H NMR spectroscopy with a gradual increase in the number of equivalents of pIBX. All attempts to optimise the reaction conditions to produce the ketones **5** and **8** as the major products were unsuccessful.

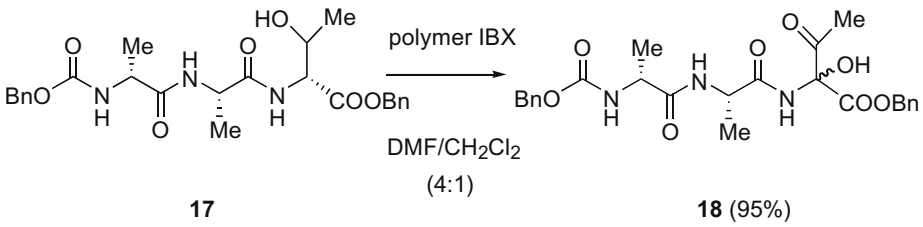
For comparison, the oxidations were also performed with IBX. As expected, similar products were obtained but additional purification was required compared with pIBX to remove the excess reagent and by-products.

In order to establish whether the oxidation at the C-α positions of **5** and **8** was specific for Thr and to investigate the stereochemistry of the reaction, the oxidation conditions were applied to Z-Val-Thr-OMe **12** (Scheme 2). The initial oxidation product(s), presumably **13** and **14** were not isolated, but the mixture was directly treated with methyl *p*-aminooxybenzoate[18] to form the corresponding oximes **15** and **16**, which were purified by chromatography.[19] As expected, the Thr H-α signal appeared as a clean doublet in **16**, while doubling of one of the methyl ester signals and the Val Me signals was consistent with loss of stereochemistry at the α-carbon and the formation of **15** as a mixture of diastereomers. There was no evidence for oxidation at the C-α position of the Val residue, confirming that the α-hydroxylation occurs only at C-α of Thr.

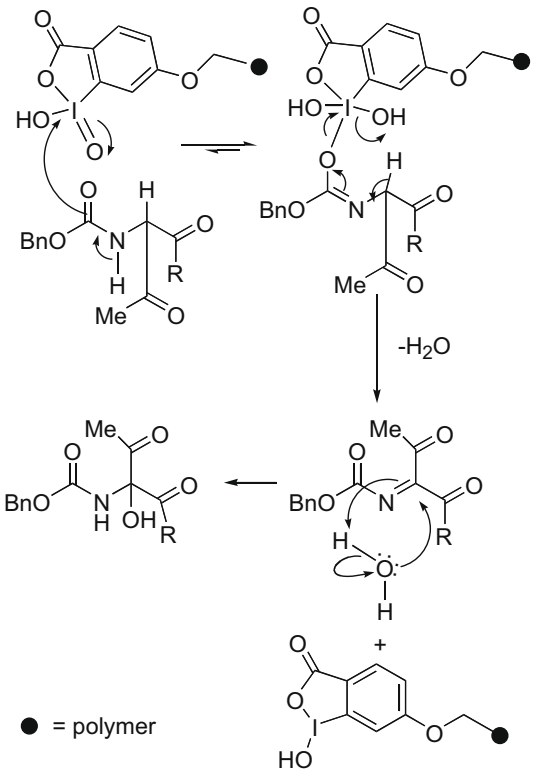
Oxidation of Z-Ala-Ala-Thr-OBn **17** (Scheme 3), the tripeptide building block present in AFGPs (Fig. 1) was performed in DMF/CH_2Cl_2 (4:1), due to the poor solubility of **17** in all organic solvents except DMF and DMSO. While 20 equiv of pIBX was required,[20] clear evidence for the formation of **18** as a mixture of diastereomers was obtained by NMR spectroscopy and high-resolution mass spectrometric analysis of the product.[21]

Hypervalent iodine reagents, including IBX and Dess–Martin periodinane, have been reported to oxidise a range of substrates including amides into imides and secondary amides to imines.[7] The mechanisms of these reactions are dependent on the exact nature of the oxidising agent and reaction conditions, and include single-electron transfer processes as well as ionic pathways.[6,7] Scheme 4 shows a proposed mechanism for the products observed in this study. The oxidation only at the α-C of Thr residues is consistent with the presence of a highly acidic C-α–proton, which is α to two carbonyl groups once the side chain is oxidised to the ketone. A recent study has reported a similar IBX-mediated α-hydroxylation of α-alkynyl carbonyl systems.[22] The alkynyl group was essential for the rapid and clean oxygen transfer from IBX at room temperature.

In summary, oxidation of Thr-containing protected peptides and polypeptides with excess pIBX provides entry to the corre-



Scheme 3.



Scheme 4.

sponding keto-peptides, which are hydroxylated at the Thr-α C position. Structure–activity studies of AFGPs have indicated that modification of the peptide backbone is tolerated,[12] and hence testing of AFGP mimics that are hydroxylated at the α-C of the glycosidated Thr residues may lead to novel new antifreeze compounds. Oxidation of Thr residues in the first generation library of Thr-containing peptides with pIBX may also be useful in combinatorial chemistry. The reaction provides a mechanism to introduce another level of diversity into the peptide library via reaction of the ketone and α-hydroxylation. In addition, the ketones can be further derivatised by reactions with Schiff bases to introduce additional diversity into the library.

Acknowledgement

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15. Selected data for **6**: Purified by flash column chromatography on silica using a gradient of EtOAc in hexane (10:90)→(60:40); yield of ketone **6** (45 mg, 87%); mp 66–68 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50–7.20 (5H, m, Ar*H*), 6.71 (1H, br s, N*H*), 5.31(1H, br s, O*H*, D_2O exchangeable), 5.10 (2H, s, ArCH_2), 3.82 (3H, s, OCH_3), 2.24 (3H, s, CH_3C(O)). ^{13}C NMR (75 MHz, $CDCl_3$): δ 197.9 (CH_3*C*(O)), 167.8 (*C*(O)OCH_3), 154.5 (*C*(O)NH), 135.4, 128.5, 128.3, 128.1 (*CAr*), 83.9 (NC(OH)C(O)), 67.4 (OCH_2Ar), 54.1 (OCH_3), 23.0 (CH_3). MS (ESI) *m/z* 304 (M+Na$^+$, 100%). HRMS: calcd for $C_{13}H_{15}NO_6Na$ 304.0792 found 304.0797.
16. Four cycles of column chromatography on silica using Et_2O:EtOAc:hexane (6:3:1) permitted isolation of a small amount of **5** for characterisation. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50–7.20 (5H, m, Ar*H*), 5.96 (1H, d, *J* 5.7 Hz, N*H*), 5.20–5.00 (3H, m, ArCH_2, *CH*), 3.81 (3H, s, OCH_3), 2.37 (3H, s, CH_3C(O)). (ESI) *m/z* 288 (M+Na$^+$, 100%). HRMS: calcd for $C_{13}H_{15}NO_5Na$ 288.0848, found 288.0844.
17. Selected data for **8** and **9**: ketone **9** (47 mg, 51%); mp 95–97 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50–7.20 (10H, m, Ar*H*), 6.92 (1H, br s, N*H*CH_2Ar), 6.68 (1H, br s, N*H*C(OH)C(O)), 5.42 (1H, br s, OH), 5.11 (2H, s, ArCH_2), 4.42 (2H, d, *J* 5.6 Hz, NHCH_2Ar), 2.28 (3H, s, CH_3C(O)). ^{13}C NMR (75 MHz, $CDCl_3$): δ 201.5 (CH_3*C*(O)), 165.7 (*C*(O)NH), 136.6, 135.5, 128.7, 128.5, 128.3, 128.1, 127.8, 127.4 (*CAr*), 85.8 (NHC(OH)), 67.4 (OCH_2Ar), 44.1 (NHCH_2Ar), 22.8 (CH_3). (ESI) *m/z* 379 (M+Na$^+$, 100%). HRMS: calcd for $C_{19}H_{20}N_2O_5Na$ 379.1270, Found 379.1271. Ketone **8** isolated as a colourless oil (22 mg, 25%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.20 (10H, m, Ar*H*), 6.70 (1H, br s, N*H*), 6.26 (1H, br s, N*H*), 5.12 (2H, s, ArCH_2), 4.85 (1H, d, *J* 5.3 Hz, *CH*), 4.42 (2H, d, *J* 5.6 Hz, NHCH_2Ar), 2.35 (3H, s, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 203.4 (CH_3*C*(O)), 165.2 (*C*(O)NH), 156.7 (*C*(O)NH), 137.5, 136.2, 129.2, 129.0, 128.8, 128.6, 128.1, 128.0 (*CAr*), 68.2 (OCH_2Ar), 65.8 (*CH*), 44.3 (NHCH_2Ar), 27.8 (CH_3). (ESI) *m/z* 363 (M+Na$^+$, 100%). HRMS: calcd for $C_{19}H_{20}N_2O_4Na$ 363.1321, found 363.1314.
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19. *Selected data for* **15** and **16**: Less polar oxime **16** (7 mg, 10%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.00 (2H, d, *J* 8.0 Hz, Ar*H*), 7.40–7.30 (5H, m, Ar*H*), 7.17 (2H, d, *J* 9.0 Hz, Ar*H*), 7.02 (1H, br d, *J* 7.1 Hz, N*H*), 6.92 (1H, br d, *J* 8.2 Hz, N*H*), 5.32 (1H, d, *J* 7.5 Hz, C*H*), 5.10 (2H, s, CH_2), 4.20–4.00 (1H, m, NHC*H*CH(CH_3)$_2$), 3.89 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 2.30–2.15 (1H, m, C*H*(CH_3)(CH_3)), 2.12 (3H, s, CH_3C=N), 1.01–0.91 (6H, m, CH(CH_3)(CH_3)). (ESI) *m/z* 536 (M+Na$^+$, 100%). HRMS: calcd for $C_{26}H_{31}O_8N_3Na$ 536.2003; found, 536.2004. More polar oxime **15** isolated as a colourless oil (27 mg, 38%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17 (1H, br s, N*H*), 8.08 (1H, br s, N*H*), 8.01 (2H, dd, *J* 9.0, 2.2 Hz, Ar*H*), 7.50–7.30 (5H, m, Ar*H*), 7.21 (2H, d, *J* 8.3 Hz, Ar*H*), 5.35–5.20 (1H, m, O*H*), 5.13 (2H, s, CH_2), 4.20–4.10 (1H, m, NHC*H*CH(CH_3)$_2$), 3.90 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 2.40–2.20 (1H, m, C*H*(CH_3)(CH_3)), 2.08 (3H, s, CH_3C=N), 1.10–0.90 (6H, m, CH(CH_3)(CH_3)). (ESI) *m/z* 552 (M+Na$^+$, 85%). HRMS: calcd for $C_{26}H_{31}O_9N_3Na$ 552.1958; found 552.1964.
20. pIBX was purchased from Merck and was used as provided. A different batch of pIBX, supplied with the same resin loading, showed less reactivity and hence more equivalents of pIBX and longer reaction times were required for the reaction to go to completion.
21. Mass spectroscopy data for **18** (ESI) *m/z* 522 (M+Na$^+$, 23%). HRMS: calcd for $C_{25}H_{29}N_3O_8Na$ 522.1852, found 522.1854.
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