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Oxidative cyclization and enzyme-free deiodination of thyroid hormones†

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We introduce the first non-enzymatic deiodination of thyroid hormones from a so far unknown hypervalent iodaoxinium state. After developing oxidative processes for thyroxine (**T4**)-derived model cyclic diaryliodonium salts, we successfully produced an iodaoxinium salt through the direct oxidation of *O*- and *N*-protected **T4**. DFT calculations revealed a novel halogen bonding-based deiodination mechanism, circumventing the traditional selenium-dependent pathways. Our findings open new avenues in thyroid hormone chemistry, suggesting alternative mechanisms for their involvement in metabolic processes, regulation of oxidative stress, and gene expression.

Introduction

Thyroid hormones (TH) play a vital role in regulating metabolic processes and are crucial for the development and metabolic homeostasis of higher organisms.[1] They are in particular capable of modulating gene expression through binding to TH-specific transcription factors, which are present in almost all vertebrate tissues.[1–3] From thyroxine (**T4**) as the main TH initially secreted by the mammalian thyroid gland, other THs such as 3,5,3′-triiodothyronine (**T3**) or 3,3′,5′-triiodothyronine (**rT3**) are formed *via* selective deiodination by one of three selenoenzymes DIO1, DIO2 and DIO3 (Scheme 1).[4–6] Of all THs, **T3** is the most active derivative with up to 40 times higher affinity to thyroid receptors than its parent compound **T4**.[7,8]

The exact mechanism of the reductive deiodination is still under investigation, but it is evident that selenocysteines are present in all related enzymes and recent literature has shown that halogen bond activation plays an important role in this process.[9–12] Nobody described a selenium-independent deiodination mechanism so far in biological systems that results in a regioselective deiodination.

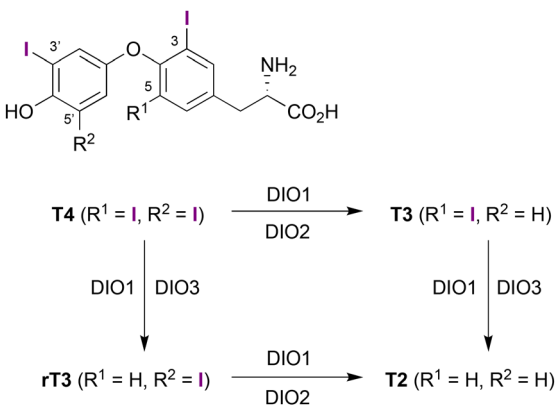
We found such a potential pathway by serendipity as part of an ongoing research program that is intended to elaborate and understand a potential biologically relevant non-enzymatic oxidation of thyroxines to hypervalent iodine reagents **ox-T3/T4** or cyclic iodaoxinium salts **1** (**cyclo-ox-T3/T4**) derived from them (Scheme 2).[13–18] In the future these substrates could also enable diverse further derivations based on previous work by Chen and co-workers.[19,20]

Results and discussion

In our initial experiments, we oxidized commercially available thyroxine (**T4**) using common oxidants such as peracids, hypochlorite solutions, SelectFluor®, and Oxone®. However, these attempts only led to the decomposition of the starting material.

Notably, the solubility issues and cleavage of the central diaryl ether, due to quinone formation, posed significant challenges.

Consequently, we shifted our focus from THs to simplified structures, where the α-amino acid is replaced with a simple methyl group and the free phenol group is protected as a



Scheme 1 Formation of different THs *via* deiodination of thyroxine (**T4**).

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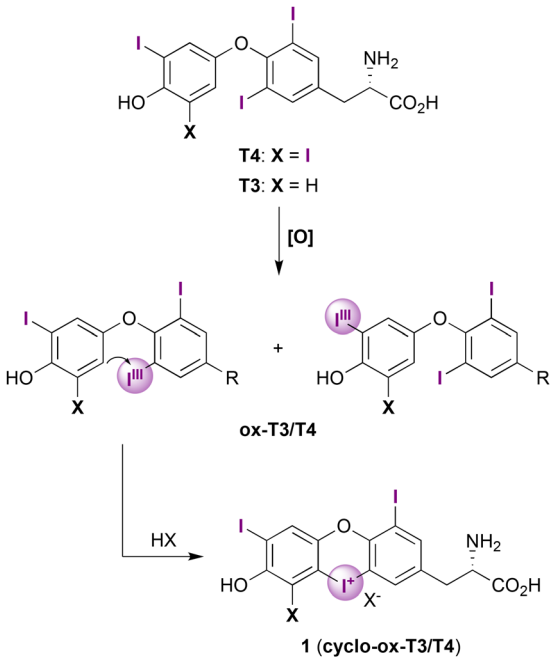


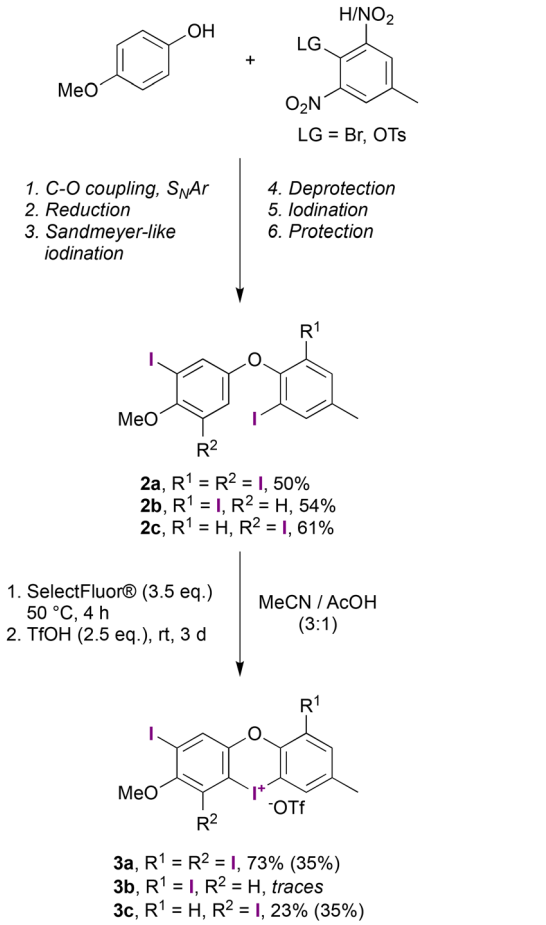
Scheme 2 Proposed oxidation of THs to hypervalent iodine reagents and their cyclization to iodaoxinium salts **1**.

methyl ether. We employed a straightforward reaction sequence, beginning with a nucleophilic aromatic substitution followed by a Sandmeyer iodination. This approach enabled us to isolate the desired simplified TH analogues **2a–c** in yields of 50–61%, starting from 4-methoxyphenol and the corresponding nitroarenes, as shown in Scheme 3. We then investigated their oxidation using literature-known conditions.[18,21,22]

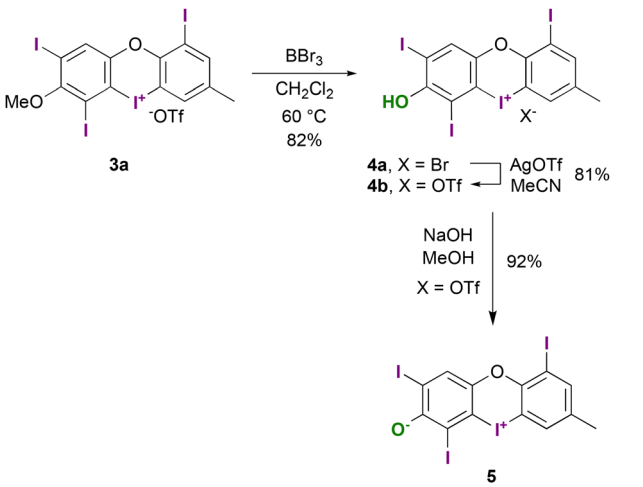
In our initial oxidation experiments, as outlined in Scheme 2, we aimed to isolate an acyclic aryl-λ^3-iodane derivative from either **ox-T3** or **ox-T4**. However, these attempts were unsuccessful. Instead, when using SelectFluor® as the oxidant and subsequently treating the reaction with TfOH, we successfully isolated the desired cyclic diaryliodonium salts, **3a** and **3c**. These compounds were obtained in yields of 73% and 23%, respectively.

Treating compound **2b** under similar oxidative conditions proved more challenging, as we only detected trace amounts of product **3b** and its isomers *via* HPLC-MS. Attempts with other polyiodinated diaryl ethers did not yield any products, as detailed in the ESI, Table 1.† Our subsequent objective was to synthesize the free phenol of type **4**, as depicted in Scheme 4. A recently published one-step method has demonstrated the stability of similar acyclic iodonium salts,[23] yet only one *meta*-hydroxy-substituted derivative closely related to our target structure has been described.[24]

For the deprotection of the methyl ether, we found that typical conditions using BBr_3 were effective. However, due to the low solubility of **3a** and its bromide salt, we had to employ elevated temperatures. Ultimately, we successfully obtained the unprotected bromide salt **4a** in 82% yield. We then



Scheme 3 Synthesis of iodoarenes **2** and their oxidative cyclization to iodaoxinium salts **3** (50 μmol scale). Yields in brackets are upscaled (500 μmol). A detailed synthetic procedure is shown in the ESI.†

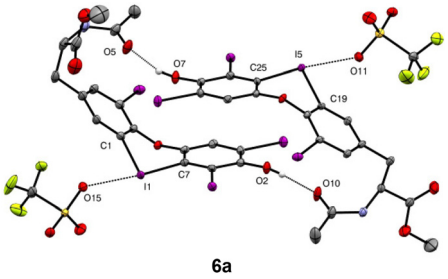


Scheme 4 Deprotection of **3a** towards **4a** and subsequent anion exchange to the triflate salt **4b**, followed by deprotonation to the zwitterion **5**.

achieved anion exchange to the triflate salt **4b** using AgOTf with an 81% yield. Further deprotonation with NaOH gave the orange-coloured zwitterion **5** with a 92% yield.

After confirming the stability of cyclic iodaoxinium salts **4**, we confidently proceeded to work with thyroid hormones (THs) directly. We began with thyroxine, converting its carboxylic acid into a methyl ester and acylating both the amino and phenol groups. However, using our previously developed method, we only observed trace amounts of the desired product. Switching to well-established oxidative conditions with *m*CPBA and TfOH proved successful. This approach enabled the direct synthesis of the iodaoxinium salt **6a** through *in situ* deprotection of the phenol group, yielding a moderate efficiency with an isolated yield of 53% (as shown in Scheme 5). We also noted that phenol **6** could be deprotonated to produce the zwitterion 7, which was stable at room temperature in its solid form achieving a yield of 72%, although decomposition was observed in highly polar solvents such as DMSO, DMF, and NMP.

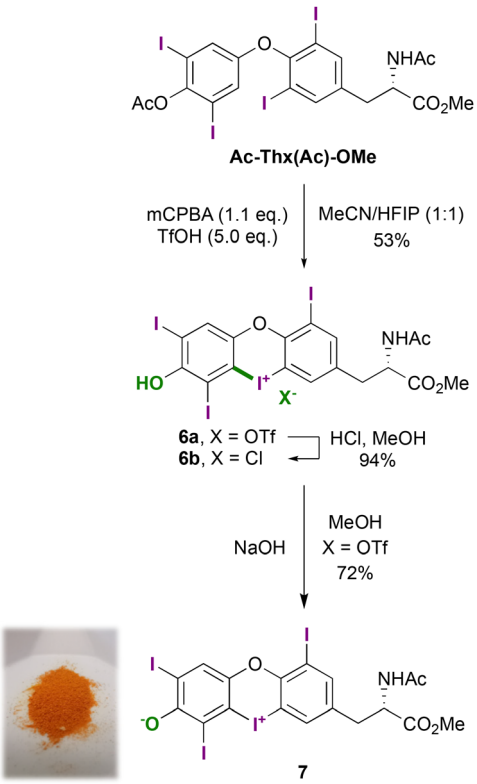
The solid state structure of the iodaoxinium salt **6a** was determined by X-ray diffraction analysis, revealing it had crystallised as a hydrogen-bridged dimer (Fig. 1). The geometry around the iodonium centre is as expected in a T-shape with C–I–C angles of 90.0° and 89.7° and triflates coordinating at angles of 175.2° and 172.69°. The I–O bond between triflate and iodine is at 2.757 Å and 2.752 Å, comparable to known iodaoxinium salts.[22] Interestingly the dimer shows hydrogen bonding between the phenol and the amide carbonyl indicated by O–O distances of 2.563 Å and 2.606 Å. We also observed significant π–π-interactions between the two phenol-bearing aromatic rings with a centroid-to-centroid distance of 3.650 Å.



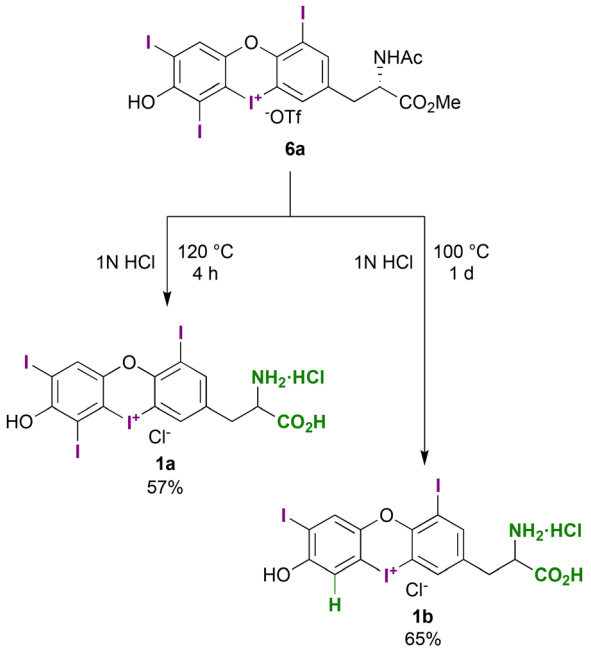
6a

Fig. 1 Single crystal structure (ORTEP drawing; hydrogen atoms, except those participating in hydrogen bonding, were omitted) of **6a** as a dimer (CCDC 2291802†) with aromatic ring interaction and hydrogen bonding visible inside the dimer. Thermal ellipsoids displayed with 50% probability. Selected parameters [Å, °]: I1–O15: 2.757(2); I5–O11: 2.752 (2); O5–O7: 2.563(4); O2–O10: 2.606(4); C1–I1–C7: 90.0(1); C19–I5–C25: 89.7(1); C7–I1–O15: 175.2(1); C25–I5–O11: 172.69(9).

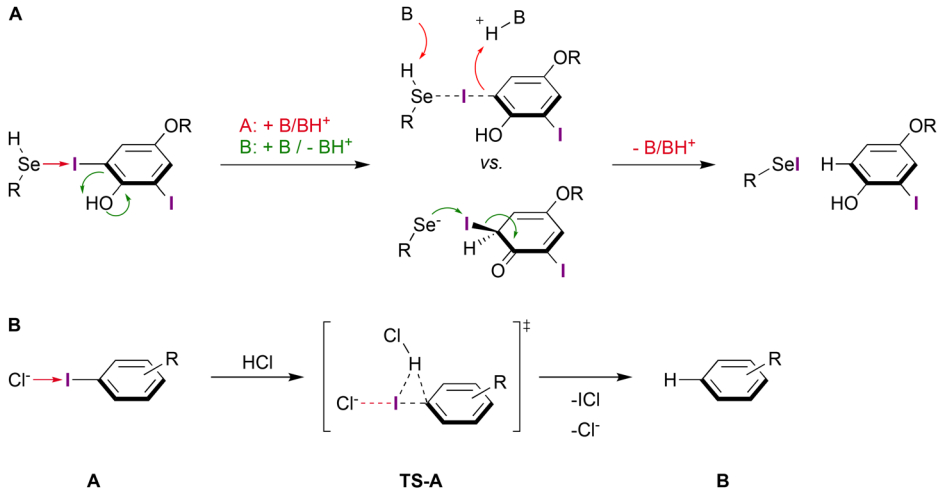
Our objective was to create a thyroid hormone (TH)-derived substrate, with the sole modification being a cyclized iodonium centre. To achieve this, we needed to deprotect the amino acid. Treating **6a** with aqueous HCl at 120 °C led to the formation of the desired **T4**-derived iodaoxinium salt **1a**, with a yield of 57% (Scheme 6). However, during a side-product analysis of this reaction using HPLC-MS, we unexpectedly discovered a deiodination product. On lowering the reaction temperature and increasing the reaction time, this side product was formed exclusively. We identified this compound as the **T3**-derived iodaoxinium salt **1b**, which was isolated in 65% yield.



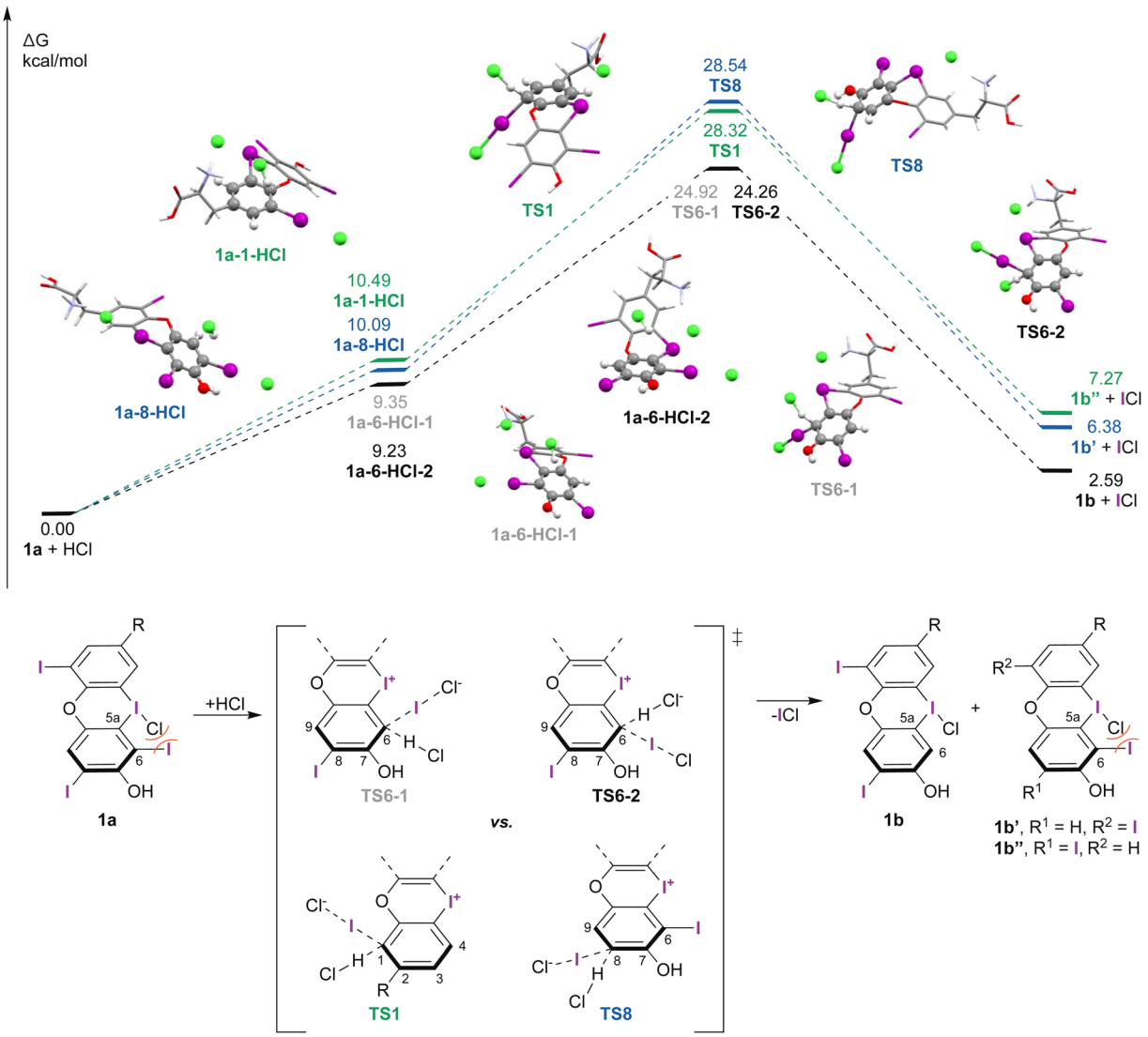
Scheme 5 Synthesis of TH-derived iodaoxinium salts **6** and **7** from protected thyroxine **T4**.



Scheme 6 Deprotection of the amino acid functionality of salt **7a** to generate the salt **1a** and *via* selective protic deiodination **1b**.



Scheme 7 A: Selenocysteine-mediated deiodination mechanism as proposed by Rafferty (red) *vs.* the mechanism *via* enolization (green). B: Corresponding chloride/HCl-mediated deiodination mechanism.



Scheme 8 Energy profile for the deiodination of **1a** *via* the addition of HCl to generate **1b**, **1b'** and **1b''**. And a simplified schematic representation of the mechanism.

To gain a deeper understanding of the unexpected deiodination, especially its high selectivity for the C6 position *ortho* to the iodonium centre, we conducted Density Functional Theory (DFT) calculations. These were performed at the PBE0-D3(BJ)/def2-TZVP+CPCM level of theory, using geometries optimized at the PBE0-D3(BJ)/def2-SVP+CPCM level. For guidance in elucidating the correct reaction pathway, we referred to a study by Rafferty and co-workers. This research explored the deiodination of thyroid hormones by selenols, employing a simplified catalytic triad.[9] Following this path, in the presence of HCl as a Brønsted-acid, a trimolecular reaction through **TS-A** should be induced to generate ICl and the deiodinated arene **B** (Scheme 7).

Building on this mechanism, we explored three separate pathways, each beginning with an XB interaction between a chloride ion and one of the three monovalent iodine atoms located at positions C1, C6, and C8. These calculated reaction pathways are depicted in Scheme 8. Initially, the addition of HCl led to the formation of XB adducts **1a-6-HCl** (with an energy of +9.23 kcal mol^{-1}), **1a-8-HCl** (+10.09 kcal mol^{-1}), and **1a-1-HCl** (+10.49 kcal mol^{-1}). In our notation, the middle index (1a-X-HCl) specifies the position of the coordinated iodine. These XB complexes then transitioned into the states **TS6-1**, **TS6-2**, **TS8**, and **TS1**. The difference between **TS6-1** and **TS6-2** lies in the varying attack sites of the chloride anion. A comparison of the relative energies of these transition states revealed that **TS6-1** and **TS6-2**, leading to the formation of the **T3**-derived iodaoxinium salt, possessed the lowest activation barriers at 24.92 kcal mol^{-1} and 24.26 kcal mol^{-1}, respectively. In contrast, **TS8** and **TS1** exhibited significantly higher barriers, at 28.32 kcal mol^{-1} and 28.54 kcal mol^{-1}, respectively. Moreover, deiodination at C6 uniquely resulted in the transformation of the bent iodaoxinium **1a** into a less bent geometry **1b**, accompanied by a notable twist angle change of +6.7° between the two phenyl rings. Additionally, the dihedral angle between C6–C5a–I–Cl relaxed by −23.8° during the transition from **1a** to **1b**. Transitions to other deiodinated iodaoxinium salts **1b′** and **1b″**, did not exhibit such significant changes in ground state geometry.

To gain further insight into this reaction and to verify the proposed halogen bond interaction, we looked at the binding of compound **6a** with tetra-*n*-butylammonium chloride (TBACl). The initial addition of one equivalent of TBACl resulted in a general downfield shift, caused by anion exchange from triflate to chloride, matching spectral data for **6b**. Upon further addition of TBACl (5 eq.) only for one carbon a shift (downfield) is observed (Fig. 2). This signal likely corresponds to position C6 as there is no correpsonding ^{13}C-signal in the NMR of C6-deiodinated **1b**. When we added a huge excess of TBACl (50 eq.), further downfield shifts are observed for C6 as well as for the other two iodinated carbons (C1 and C8).

As previously mentioned, the iodaoxinium salt **1a** possesses an asymmetric bent geometry, allowing it to be protonated by HCl from either a convex or a concave face. In Scheme 9, we present a direct comparison of both pathways. Protonation through the convex face, *via* **TS6-1**, reveals a weak XB-interaction between the chlorine atom of the HCl molecule and the iodonium ion, measured at a distance of 3.22 Å and an angle of 158.49°. On the other hand, protonation from the concave face leads to a slightly more favourable transition state, **TS6-2**, with an energy difference of −0.66 kcal mol^{-1}. In this state, a shorter than van der Waals distance between the iodine atom at C6 and the iodonium ion still suggests a weak XB-bond, however with an even more distorted angle of 146.83°. This interaction appears to be energetically slightly more favourable compared to the Cl–I interaction observed in **TS6-1**. Given the minimal energy difference between the two transition states, it's likely that both pathways will occur under the applied elevated reaction temperature of 100 °C.

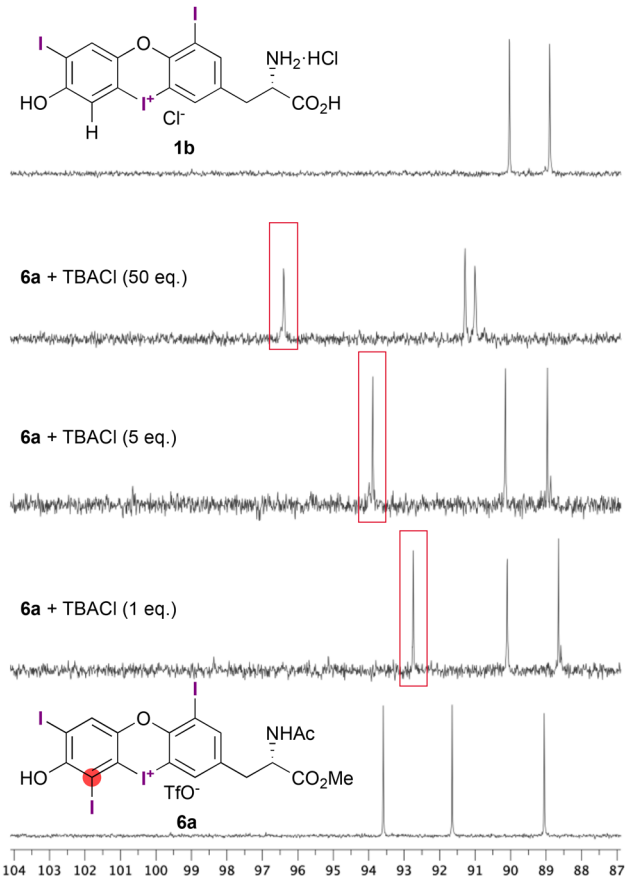
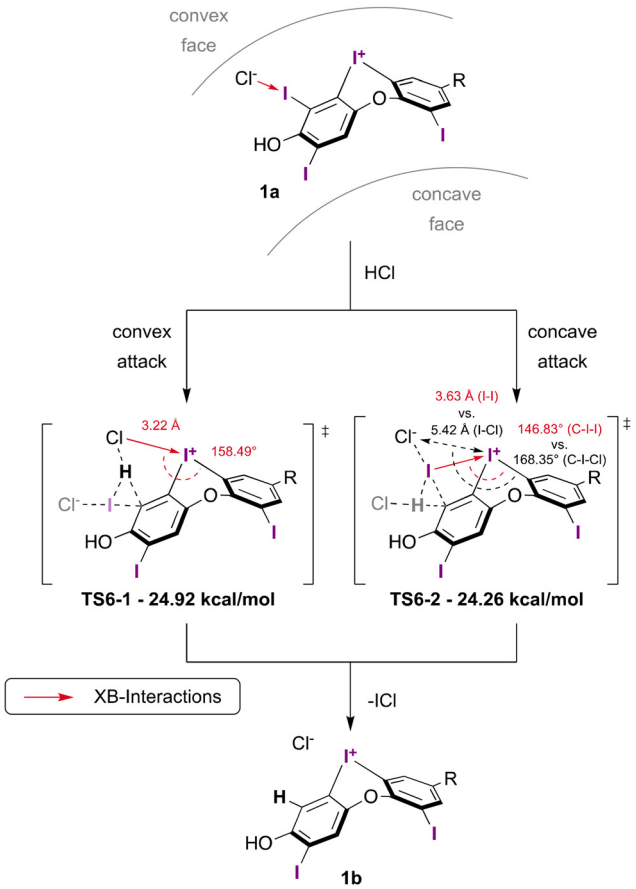


Fig. 2 NMR observations upon addition of different amounts of tetra-*n*-butylammonium chloride (TBACl) to iodaoxinium salt **6a** and comparison to **1b**.

However, both transition states represent a unique selenoenzyme-free deiodination mechanism of thyroxines based on intrinsic XB interactions. This discovery not only broadens the understanding of thyroid hormone chemistry but also challenges the conventional belief that enzymatic pathways are the sole mediators for the selective deiodination of THs. These XB interactions, driving the deiodination process, highlight a novel aspect of thyroxine's molecular behaviour, potentially reshaping our approach to thyroid hormone-related (bio) chemistry.



Scheme 9 Distinct transition states from a convex and concave protonation of **1a**, both leading to **1b**.

Conclusions

In summary, our research demonstrates that oxidized thyroxine (**T4**), in the form of a hypervalent iodaoxinium salt (**1a**), is a stable entity that can be directly synthesized from *O*-acylated thyroxine under oxidative and acidic conditions. Interestingly, we discovered that **1a** can undergo selective deiodination at the C6 position, resulting in an oxidized **T3**-derivative. This deiodination process, which occurs without the involvement of selenoenzymes, is not only unprecedented but also features a unique activation mechanism driven by XB interactions with the central iodonium ion. To further explore the possibility of these thyroxine-derived iodaoxinium salts acting as reactive intermediates under oxidative stress in biological systems, it is essential to confirm their presence *in vivo*. Additionally, we aim to investigate their potential reduction to T3 or reverse T3 (rT3) by natural reducing agents, such as flavins or dihydropyridines.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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