Synthesis, Stability, and Biological Studies of Fluorinated Analogues of Thromboxane A2

Mallah, S

DOI 10.1021/acscentsci.0c00310
ISSN 2374-7951

Publisher: ACS Central Science

ACS central science

http://pubs.acs.org/journal/acscii

Research Article

Synthesis, Stability, and Biological Studies of Fluorinated Analogues of Thromboxane A_2

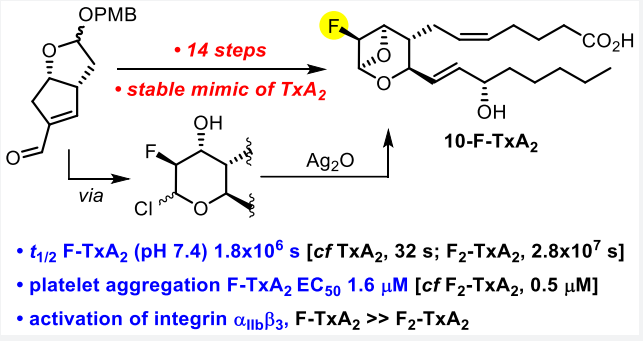
Changcheng Jing, Shahida Mallah, Ella Kriemen, Steven H. Bennett, Valerio Fasano, Alastair J. J. Lennox,* Ingeborg Hers,* and Varinder K. Aggarwal*

Cite This: *ACS Cent. Sci.* 2020, 6, 995−1000

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Platelet activation results in the generation of thromboxane A_2 (TxA_2), which promotes thrombus formation by further amplifying platelet function, as well as causing vasoconstriction. Due to its role in thrombus formation and cardiovascular disease, its production is the target of antiplatelet drugs such as aspirin. However, the study of TxA_2-stimulated cellular function has been limited by its instability ($t_{1/2}$ = 32 s, pH = 7.4). Although more stable analogues such as U46619 and difluorinated 10,10-F_2-TxA_2 have been prepared, we targeted a closer mimic to TxA_2 itself, monofluorinated 10-F-TxA_2, since the number of fluorine atoms can affect function. Key steps in the synthesis of F-TxA_2 included α-fluorination of a lactone bearing a β-alkoxy group, and a novel synthesis of the strained acetal. F-TxA_2 was found to be 10^5 more stable than TxA_2, and surprisingly was only slightly less stable than F_2-TxA_2. Preliminary biological studies showed that F-TxA_2 has similar potency as TxA_2 toward inducing platelet aggregation but was superior to F_2-TxA_2 in activating integrin $\alpha_{IIb}\beta_3$.



1. INTRODUCTION

Thromboxane A_2 (TxA_2) is produced enzymatically from arachidonic acid through the action of several enzymes including cyclooxygenase (COX) and thromboxane synthase in response to tissue injury, promoting hemostasis, vasoconstriction, and wound healing.[1−3] However, these necessary features for survival can also cause death to those susceptible to or suffering from cardiovascular disease (CVD).[4−9] Current first-line therapy involves the use of nonsteroidal anti-inflammatory drugs (NSAIDs) which block >95% of COX1 activity and therefore TxA_2 production.[10] However, the treatment suffers from side effects associated with shutting down the whole prostanoid cascade and with resistance in some patient groups.[11]

The study of TxA_2 has been limited by its high instability ($t_{1/2}$ = 32 s, pH = 7.4)[1] and so a number of more stable analogues have been prepared in which one or both oxygens of the strained acetal have been replaced by carbon,[12] sulfur,[13] or a less strained bicyclic structure (e.g., U46619, Figure 1).[14,15] A different strategy is to retain the strained acetal but reduce the rate of hydrolysis by incorporating either bromine[16] or, more importantly, fluorine[17−20] atoms at the C-10 position (Figure 1). Although the synthesis[21,22] of monofluorinated F-TxA_2 **1** has been attempted,[23] only the difluoro analogue **2** has succumbed to total synthesis,[17] which showed similar potency in platelet aggregation to the parent compound.[20] The stability of **2** has only been investigated using a model compound (**3**), which, as expected, showed much higher stability than TxA_2 ($t_{1/2}$ > 30 days, pH = 7.4).[20] We were interested in targeting F-TxA_2 **1** since the number of fluorine atoms can have a significant impact on function. For example, in a comparative study of the CHF-

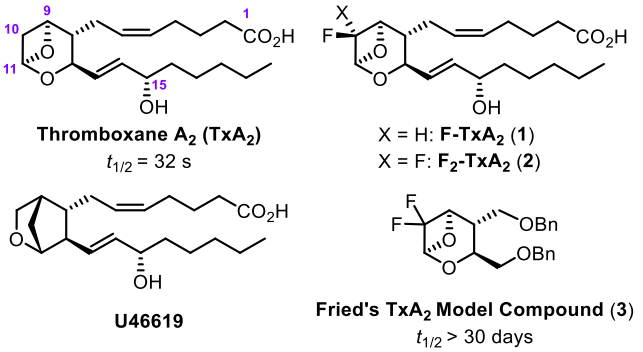


Figure 1. Thromboxane A_2 and its analogues.

Received: March 17, 2020
Published: June 10, 2020

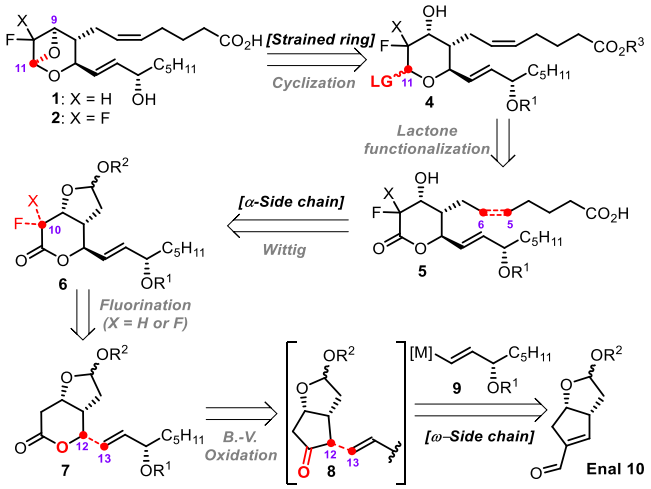
https://dx.doi.org/10.1021/acscentsci.0c00310
ACS Cent. Sci. 2020, 6, 995−1000

and CF_2- phosphonate analogues of *sn*-glycerol-3-phosphate, O'Hagan found that the monofluorinated was better than the difluorinated substrate for the dehydrogenase enzyme.[24−26] We now report the first synthesis of F-TxA_2 **1** and compare its stability and biological activity with that of F_2-TxA_2 **2**.

2. RESULTS AND DISCUSSION

Our retrosynthetic analysis of F-TxA_2 is shown in Scheme 1. We envisioned forming the strained acetal by an intramolecular

Scheme 1. Retrosynthesis of Fluorinated Thromboxanes from Bicyclic Enal

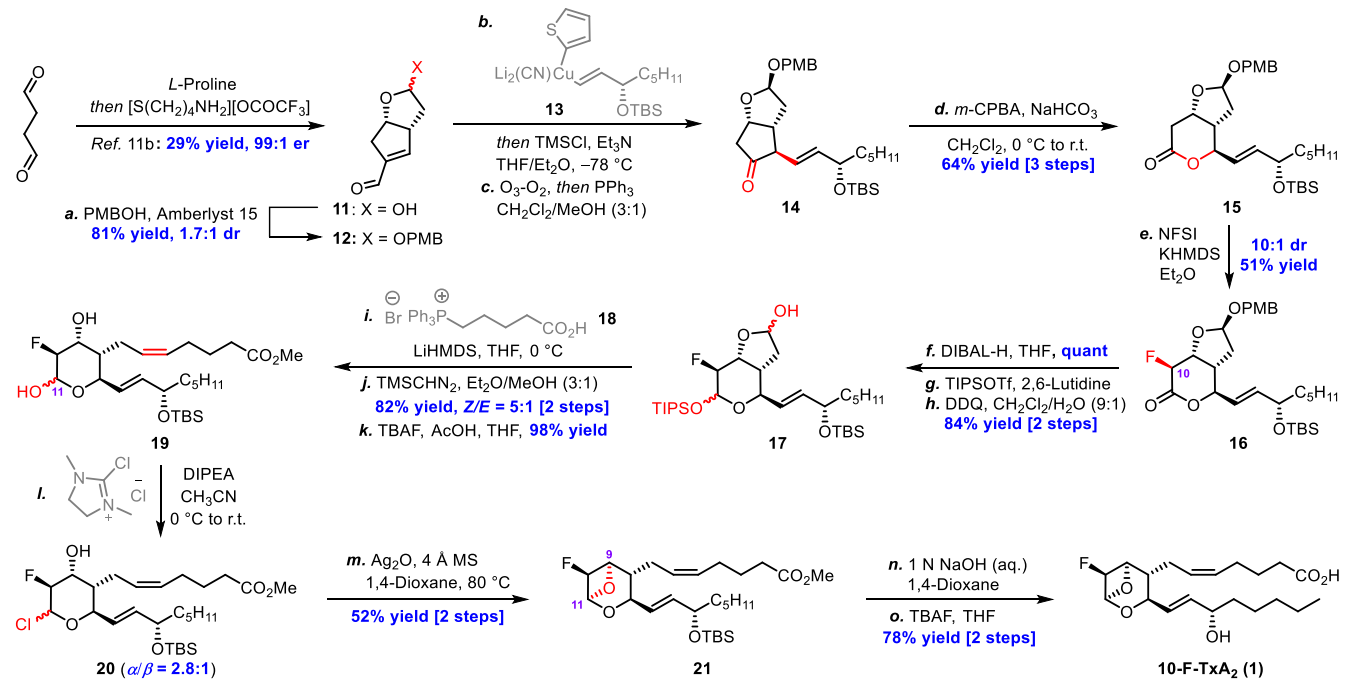


cyclization and introducing the upper side chain by a Wittig reaction on the corresponding fluorinated lactol. Lactone **6** could be obtained by fluorination of the enolate of lactone **7**, which itself could be synthesized by Baeyer−Villiger oxidation of ketone **8**. Ketone **8** could then be obtained from conjugate addition of the lower side chain **9** to our key enal intermediate **10** followed by ozonolysis. At the outset, the main challenges presented in the synthesis were formation of the strained acetal and fluorination of the enolate bearing a potential leaving group at the β-position.

Synthesis of Fluorinated Thromboxane A_2. Our synthesis began from PMB-acetal **12**, available in 3 steps in high er using our established proline-catalyzed aldol dimerization of succinaldehyde (Scheme 2).[27,28] Initially, we elected to carry through the major β-isomer of the acetal to simplify analysis. Conjugate addition of the mixed vinyl cuprate **13** followed by trapping with TMSCl and ozonolysis[27] gave ketone **14** which was converted into the key lactone intermediate **15** through a Baeyer−Villiger oxidation[29,30] (64% yield, over 3 steps).

With a scalable synthesis of lactone **15** in hand, we embarked on the fluorination reaction. Lactones bearing siloxy and benzyloxy groups in the β-position are particularly prone to elimination upon deprotonation and have to be trapped by reactive electrophiles at low temperature.[31−35] Initial investigation showed that NFSI was a sufficiently reactive electrophile, and after optimization we found that the reaction proceeded with good selectivity (10:1 dr) and yield (51%) using 1.2 equiv KHMDS and 2.5 equiv NFSI in Et_2O. Following PMB deprotection with DDQ, we explored the Wittig reaction with (4-carboxybutyl)triphenyl-phosphonium bromide (**18**), but this invariably led to intractable mixtures. We suspected that the lactone was interfering in this step and so converted lactone **16** into siloxyacetal. This time, following PMB deprotection, Wittig reaction using phosphonium salt **18** with *t*-BuOK surprisingly gave the corresponding epoxide in 67% yield.[36] To avoid epoxide formation, we screened alternative conditions and found that using LiHMDS with a ratio of hemiacetal (**17**):Wittig salt:LiHMDS of 1:4:8 at 0 °C gave the corresponding alkene in 82% yield as a separable 5:1 mixture of *Z*/*E* isomers after esterification with $TMSCHN_2$. Selective desilylation of the TIPS group with TBAF/AcOH gave the required lactol **19** in 98% yield.[37−41]

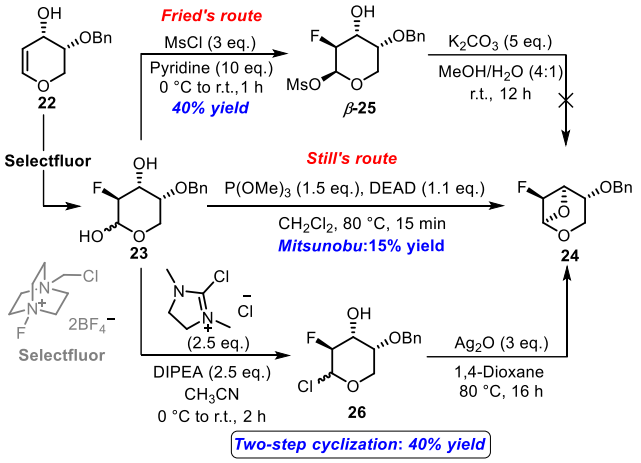
Scheme 2. Synthesis of Key Lactone Precursor and Completion of the Synthesis of the Monofluorinated Thromboxane A_2



https://dx.doi.org/10.1021/acscentsci.0c00310
ACS Cent. Sci. 2020, 6, 995−1000

To complete the synthesis of 10-F-TxA_2, we required a method for the construction of the strained acetal. Owing to its known sensitivity and the low yields previously obtained for the construction of this motif, we decided to explore this key step on model substrate **23**. This was prepared from D-arabinal-derived glycal **22** by fluorination with selectfluor (Scheme 3).[42−44] Two

Scheme 3. Formation of Strained Acetal on Model Hemiacetal 23



methods for making the strained acetal had been reported previously, Still's Mitsunobu reaction[45,46] and Fried's displacement of the mesylate,[17−20] but neither was successful on hemiacetal **23** as shown in Scheme 3. These synthetic hurdles required us to find a new method to make the strained acetal. Shoda reported that treatment of unprotected glycopyranoses with 2-chloro-1,3-dimethylimidazolinium chloride (DMC) gave the corresponding 1,6-anhydro sugars directly.[47,48] This reagent was tested on hemiacetal **23**, but although we did not obtain the desired acetal **24** directly, we did isolate chloride **26** with complete chemoselectivity. The fortuitous formation of the (unstable) chloride presented another opportunity, since glycosyl chlorides can be activated by silver salts to promote their displacement.[49] Indeed, treatment with Ag_2O promoted cyclization giving the acetal **24** in 40% yield, providing a novel solution to the synthesis of strained acetals.

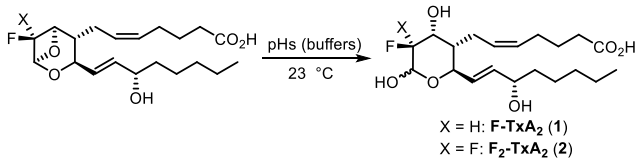
Moving onto the real target, brief optimization of the chlorination/cyclization steps was again required but optimum conditions were quickly established. Treatment of hemiacetal **19** with 6 equiv of each of the chlorination reagent, DIPEA, and Ag_2O gave the desired acetal **21** in 52% yield (Scheme 2). Finally, hydrolysis of **21** with 1.0 N NaOH in 50% 1,4-dioxane/water followed by deprotection with TBAF furnished F-TxA_2 **1** in 78% yield.

We also tried to prepare the other diastereoisomer 10α-F-TxA_2 from the minor diastereomer formed in the fluorination of lactone **15**. While we were able to carry this diastereoisomer through to the corresponding diol (hydroxy hemiacetal, diastereomer of **19**), attempts to prepare the chloride and the subsequent cyclization were thwarted by competing elimination and hydrolysis.

By adapting this strategy, we were able to prepare F_2-TxA_2 (see Supporting Information), so that its stability and biological activity could also be assessed. With both fluorinated TxA_2 analogues in hand, we were then able to compare their stabilities with the parent TxA_2 and study their biological activity.

Stability Studies of Fluorinated Thromboxane A_2 and Model Compounds. The hydrolytic stability of TxA_2 at pH 7.4 (37 °C) was measured and found to have a $t_{1/2}$ of 32 s.[1] Fried measured the stability of his F_2-TxA_2 model compound **3**, which is similar in structure to F_2-TxA_2, at pH 1.27 (22 °C) to have a $t_{1/2}$ of 86 min. While this 10^8 difference in rate constant is interesting to note, the difference in pH and temperature of these measurements renders a direct comparison of stability, and an assessment of the effect of fluorine, very difficult. Hence, we sought to compare the stability of TxA_2 with its fluorinated analogues by measuring the kinetics of hydrolysis under the same conditions. Using ^{19}F NMR to monitor the decay of the acetal moiety, we determined pseudo first-order rate constants for the hydrolysis of **1** and **2** (Table 1) under buffered

Table 1. Kinetics of Hydrolysis.[a]



compound	pH	k_1' (s^{-1})	$t_{1/2}$
F-TxA_2 (**1**)	7.40	3.93×10^{-7}	20 days
	2.42	8.93×10^{-5}	2.2 h
F_2-TxA_2 (**2**)	7.40	2.5×10^{-8}	46 weeks
	2.42[b]	1.01×10^{-6}	190 h
	1.80	5.46×10^{-5}	3.5 h
	1.25	1.80×10^{-4}	64 min

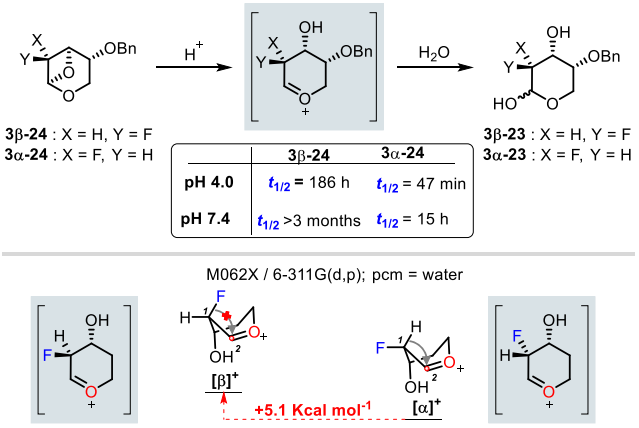
[a]Hydrolyses of **1** and **2** were measured under buffered conditions (50 mM), using ^{19}F NMR to monitor the decay of the ketal. k_1' = pseudo first-order rate constants. $t_{1/2}$ = half-life. [b]Average of two runs.

conditions. At pH 7.4, we found that F-TxA_2 (**1**) has a half-life of 20 days, which is 10^5 more stable than TxA_2. Interestingly, F_2-TxA_2 (**2**) was only 1 order of magnitude more stable at pH 7.4 with a half-life of over 40 weeks. We then measured hydrolysis rates of **1** and **2** at lower pHs (Table 1), where, as expected, decreasing the pH decreased the stability. The rate of hydrolysis we measured for F_2-TxA_2 (**2**) at pH 1.25 ($t_{1/2}$ = 64 min) was in good agreement with that of Fried's model compound **3** at pH 1.27 ($t_{1/2}$ = 86 min).[20,50]

The marginal increase in stability of **2** compared to **1** at pH 7.4 was unexpected, as the increase in stability caused by inductive effects of the electronegative fluorine atoms is usually additive.[51,52] Thus, we speculated that there might be a strong stereoelectronic effect governing the stability of the strained acetal. Unfortunately, we were not able to prepare 10α-F-TxA_2 to test this, so we compared the stability of the two diastereoisomers of model compound **24** (3α-**24** with 3β-**24**, Scheme 4). Indeed, we measured a very substantial difference in hydrolysis rate between the isomers: 3β-**24** was ca. 200× more stable than 3α-**24**. The greater lability of 3α vs 3β-**24** presumably originates from having a better σ-donor (C−H vs C−F bond) aligned to the incipient oxocarbenium ion, as supported by DFT calculations on a model substrate (Scheme 4; see Supporting Information for further discussion). Our inability to make 10α-F-TxA_2 could therefore be due to its greater instability. Furthermore, as **3a**-**24** exhibited a half-life of just 15 h at pH 7.4, it is likely that 10α-F-TxA_2 would not have been suitable for biological studies (see Supporting Information for full details). These studies therefore reveal that the stability derived from the

https://dx.doi.org/10.1021/acscentsci.0c00310
ACS Cent. Sci. 2020, 6, 995−1000

Scheme 4. Investigations into Hydrolysis of Model Compound 24



stereoelectronic effect of an antiperiplanar fluorine is very significant compared to a syn-periplanar fluorine and provides a rationale for the nonadditive inductive effect of fluorine atoms on acetal hydrolysis.

Biological Studies. To evaluate the biological activity of the fluorinated thromboxanes **1** and **2**, concentration−response experiments were performed on human platelets, and platelet aggregation was recorded by light transmission aggregometer. The stable PGH_2 analogue U46619 has been used widely as a standard of comparison for evaluating TxA_2-like activity and so was included in this study.[53−56] Concentration−response curves were fitted (Figure 2) and EC_{50} values were calculated (Table 2). The data show that F-TxA_2 has similar activity as U46619 in inducing platelet aggregation but is almost 3-fold less potent than F_2-TxA_2. While F_2-TxA_2 was more potent, the E_{Max} was significantly lower than U46619 and F-TxA_2, suggesting partial agonism at TxA_2 receptors. As platelet amplification pathways such as ADP release and integrin $\alpha_{IIb}\beta_3$ outside-in signaling can potentially mask a weaker agonist response in aggregation experiments, we were also interested to study a more direct functional readout of platelet activation: integrin $\alpha_{IIb}\beta_3$ activation. Interestingly, we found that, in contrast to F-TxA_2 and U46619, F_2-TxA_2 induced only weak integrin $\alpha_{IIb}\beta_3$ activation (Figure 3). Since both F-TxA_2 and U46619 have similar activity in both aggregation and integrin $\alpha_{IIb}\beta_3$ activation experiments and U46619 has comparative activity to TxA_2,[23] our data strongly indicates that F-TxA_2 is a closer mimic to TxA_2 than F_2-TxA_2. Further biological and pharmacological studies are ongoing.

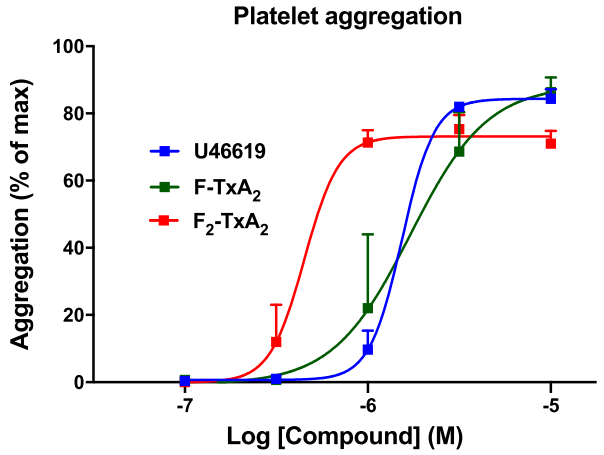


Figure 2. TxA_2-like properties of mono- and difluorinated TxA_2 analogues on platelet aggregation. Aggregation of human platelet-rich-plasma induced by U46619, F-TxA_2, and F_2-TxA_2 (average ± SEM, $n = 3$).

Table 2. Concentration of TxA_2 Analogues Which Produces 50% Of Maximal Aggregation

compound	pEC_{50}[a] ± SEM, $n = 3$	EC_{50}[b] (μM), $n = 3$	E_{Max}[c] (%) ± SEM, $n = 3$
U46619	5.85 ± 0.06	1.4	84.1 ± 2.7
F-TxA_2 (1)	5.80 ± 0.18	1.6	77 ± 13.1
F_2-TxA_2 (2)	6.30 ± 0.12	0.5	69.3 ± 4.5

[a]pEC_{50}, the negative logarithm of EC_{50}. [b]EC_{50}, the concentration of agonist that produces 50% of maximum response. [c]E_{Max}, the maximum aggregation in platelet-rich plasma.

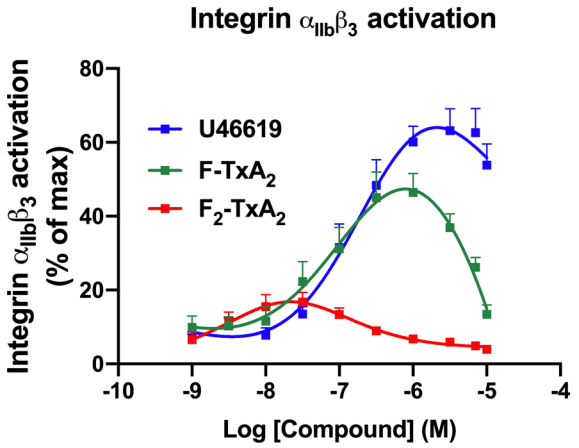


Figure 3. TxA_2-like properties of mono and difluorinated TxA_2 analogues on platelet integrin $\alpha_{IIb}\beta_3$ activation. Washed platelets were stimulated with U46619, F-TxA_2, and F_2-TxA_2 in the presence of 1 μM ADP for 15 min and integrin $\alpha_{IIb}\beta_3$ activation was determined using FITC-PAC1 by FACS analysis. Data is expressed as a percentage of the maximal α-thrombin (0.5 U/mL) response (average ± SEM, $n = 5$).

3. CONCLUSIONS

In summary, we have developed novel syntheses of chemically stable fluorinated thromboxanes, utilizing our key enal intermediate, which is readily available in high ee. The total synthesis of the F-TxA_2 and F_2-TxA_2 were completed in 17 and 18 steps, respectively, from 2,5-dimethoxytetrahydrofuran. The scalable route enabled >100 mg of advanced material (e.g., **21**) to be prepared for chemical and biological screening. In addition to overcoming some unexpected challenges associated with incorporating and carrying fluorine through a synthesis, we have also developed a new method for constructing the highly strained acetal. As expected, F-TxA_2 does indeed possess markedly greater stability than TxA_2, enabling it to be further studied in biological assays. Preliminary biological studies showed that F-TxA_2 is the closest mimic to date of TxA_2 having similar potency toward inducing platelet aggregation, and is considerably superior to F_2-TxA_2 in activating integrin $\alpha_{IIb}\beta_3$.

https://dx.doi.org/10.1021/acscentsci.0c00310
ACS Cent. Sci. 2020, 6, 995−1000

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscentsci.0c00310.

Experimental procedures and characterization data for new compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Alastair J. J. Lennox − *School of Chemistry, University of Bristol, Bristol BS8 1TS, United Kingdom;* orcid.org/0000-0003-2019-7421; Email: A.lennox@bristol.ac.uk

Ingeborg Hers − *School of Physiology, Pharmacology & Neuroscience, University of Bristol, University Walk, Bristol BS8 1TD, United Kingdom;* orcid.org/0000-0002-6467-5715; Email: I.Hers@bristol.ac.uk

Varinder K. Aggarwal − *School of Chemistry, University of Bristol, Bristol BS8 1TS, United Kingdom;* orcid.org/0000-0003-0344-6430; Email: V.Aggarwal@bristol.ac.uk

Authors

Changcheng Jing − *School of Chemistry, University of Bristol, Bristol BS8 1TS, United Kingdom;* orcid.org/0000-0002-2376-1828

Shahida Mallah − *School of Physiology, Pharmacology & Neuroscience, University of Bristol, University Walk, Bristol BS8 1TD, United Kingdom;* orcid.org/0000-0001-6669-2773

Ella Kriemen − *School of Chemistry, University of Bristol, Bristol BS8 1TS, United Kingdom*

Steven H. Bennett − *School of Chemistry, University of Bristol, Bristol BS8 1TS, United Kingdom;* orcid.org/0000-0001-8076-485X

Valerio Fasano − *School of Chemistry, University of Bristol, Bristol BS8 1TS, United Kingdom;* orcid.org/0000-0003-1819-4483

Complete contact information is available at: https://pubs.acs.org/10.1021/acscentsci.0c00310

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank EPSRC (EP/M012530/1) and the Royal Society (University Research Fellowship to A.J.J.L.) for support of this work. We thank Perry Rosen for insightful discussions and Siying Zhong and Lydia Dewis for technical assistance with NMR.

■ REFERENCES

(1) Hamberg, M.; Svensson, J.; Samuelsson, B. Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc. Natl. Acad. Sci. U. S. A.* **1975**, *72*, 2994−2998.

(2) Narumiya, S.; Sugimoto, Y.; Ushikubi, F. Prostanoid Receptors: Structures, Properties, and Functions. *Physiol. Rev.* **1999**, *79*, 1193−1226.

(3) Bhagwat, S. S.; Hamann, P. R.; Still, W. C.; Bunting, S.; Fitzpatrick, F. A. Synthesis and structure of the platelet aggregation factor thromboxane A_2. *Nature* **1985**, *315*, 511−513.

(4) Szczeklik, A.; Gryglewski, R. J.; Musial, J.; Grodzínska, L.; Serwówska, M.; Wójcik-Switek, L.; Marcinkiewiewicz, E. Arachidonic acid-induced platelet aggregation and thromboxane A_2 generation in patients with coronary heart disease. *Acta Biol. Med. Ger.* **1978**, *37*, 741−742.

(5) Lewy, R. I.; Smith, J. B.; Silver, M. J.; Saia, J.; Walinsky, P.; Wiener, L. Detection of thromboxane B_2 in peripheral blood of patients with Prinzmetal's angina. *Prostaglandins Med.* **1979**, *2*, 243−248.

(6) Zipser, R. D.; Radvan, G. H.; Kronborg, I. J.; Duke, R.; Little, T. E. Urinary thromboxane B_2 and prostaglandin E_2 in the hepatorenal syndrome: evidence for increased vasoconstrictor and decreased vasodilator factors. *Gastroenterology* **1983**, *84*, 697−703.

(7) Parelon, G.; Mirouze, D.; Michel, F.; Crastes de Paulet, P.; Chaintreuil, J.; Crastes de Paulet, A.; Michel, H. Urinary prostaglandins in the hepatorenal syndrome of cirrhotic patients: role of thromboxane A_2 and an imbalance of precursor polyunsaturated fatty acids. *Gastroenterol. Clin. Biol.* **1985**, *9*, 290−297.

(8) Nagai, H.; Shimazawa, T.; Yakuo, I.; Aoki, M.; Koda, A.; Kasahara, M. The role of thromboxane A_2 [TxA_2] in liver injury in mice. *Prostaglandins* **1989**, *38*, 439−446.

(9) Ma, H. B.; Young, M.; Yang, Y. Thromboxane (TX), Prostaglandins (PG) and Atorvastatin (Liptor) Literatures. *N. Y. Sci. J.* **2015**, *8*, 93−100.

(10) Catella-Lawson, F.; Reilly, M. P.; Kapoor, S. C.; Cucchiara, A. J.; DeMarco, S.; Tournier, B.; Vyas, S. N.; Fitzgerald, G. A. Cyclooxygenase Inhibitors and the Antiplatelet Effects of Aspirin. *N. Engl. J. Med.* **2001**, *345*, 1809−1817.

(11) Eikelboom, J. W.; Hirsh, J.; Weitz, J. I.; Johnston, M.; Yi, Q. L.; Yusuf, S. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. *Circulation* **2002**, *105*, 1650−1655.

(12) Nicolaou, K. C.; Magolda, R. L.; Smith, J. B.; Aharony, D.; Smith, E. F.; Lefer, A. M. Synthesis and biological properties of pinane-thromboxane A_2, a selective inhibitor of coronary artery constriction, platelet aggregation, and thromboxane formation. *Proc. Natl. Acad. Sci. U. S. A.* **1979**, *76*, 2566−2570.

(13) Hamanaka, N.; Ohuchida, S.; Hayashi, M. Syntheses of thromboxane A_2 analogs: Thia- and dithiathromboxane A_2. *Adv. Prostaglandin Thromboxane Leukot Res.* **1983**, *11*, 319−322.

(14) Bundy, G. L. The synthesis of prostaglandin endoperoxide analogs. *Tetrahedron Lett.* **1975**, *16*, 1957−1960.

(15) Coleman, R. A.; Humphrey, P. P. A.; Kennedy, I.; Levy, G. P.; Lumley, P. U-46619, a selective thromboxane A_2-like agonist? *Br. J. Pharmacol.* **1980**, *68*, 127−128.

(16) Nokura, Y.; Nakazaki, A.; Nishikawa, T. Synthesis of Dibromo Compounds Containing 2,6-Dioxabicyclo[3.1.1]heptane Similar to Core Moiety of Thromboxane A_2. *Heterocycles* **2018**, *96*, 127−136.

(17) Fried, J.; John, V.; Szwedo, M. J., Jr.; Chen, Ch.-K.; O'Yang, C. Synthesis of 10,10-Difluorothromboxane A_2, a Potent and Chemically Stable Thromboxane Agonist. *J. Am. Chem. Soc.* **1989**, *111*, 4510−4511.

(18) Morinelli, T. A.; Okwu, A. K.; Mais, D. E.; Halushka, P. V.; John, V.; Chen, Ch.-K.; Fried, J. Difluorothromboxane A_2 and stereoisomers: Stable derivatives of thromboxane A_2 with differential effects on platelets and blood vessels. *Proc. Natl. Acad. Sci. U. S. A.* **1989**, *86*, 5600−5604.

(19) Witkowski, S.; Rao, Y. K.; Premchandran, R. H.; Halushka, P. V.; Fried, J. Total Synthesis of (+)-10,10-Difluorothromboxane A_2 and Its 9,11 and 15 Stereoisomers. *J. Am. Chem. Soc.* **1992**, *114*, 8464−8472.

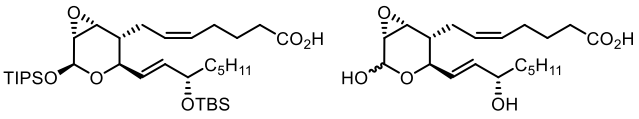
(20) Fried, J.; Hallinan, E. A.; Szwedo, M. J., Jr. Synthesis and Properties of 7,7-Difluoro Derivatives of the 2,6-Dioxa[3.1.1]-bicycloheptane Ring System Present in Thromboxane A_2. *J. Am. Chem. Soc.* **1984**, *106*, 3871−3872.

(21) For a review on the synthesis of thromboxane compounds, see: Pelyvás, I. F.; Thiem, J.; Tóth, Z. G. Access to Thromboxane Compounds: Syntheses from Carbohydrates, as Natural Chiral Pools. *J. Carbohydr. Chem.* **1998**, *17*, 1−26.

(22) For a review on the synthesis of thromboxane compounds, see: Newton, R. F.; Roberts, S. M.; Taylor, R. J. K. Strategies Employed in the Synthesis of Prostacyclins and Thromboxanes. *Synthesis* **1984**, *1984*, 449−478.

(23) Tamara, L. Studies Directed Towards the Synthesis of 10-α-Fluoro-Thromboxane A_2. Ph.D. Dissertation. University of Pennsylvania, 1986.

(24) Nieschalk, J.; O'Hagan, D. Monofluorophosphonates as Phosphate Mimics in Bioorganic Chemistry: A Comparative Study of CH_2-, CHF- and CF_2-Phosphonate Analogues of *sn*-Glycerol-3-phosphate as Substrates for *sn*-Glycerol-3-phosphate Dehydrogenase. *J. Chem. Soc., Chem. Commun.* **1995**, 719−720.
(25) For an example where the mono fluorinated compound is superior to the difluorinated compound, see: van Niel, M. B.; et al. Fluorination of 3-(3-(Piperidin-1-yl)propyl)indoles and 3-(3-(Piperazin-1-yl)propyl)indoles Gives Selective Human 5-HT_{1D} Receptor Ligands with Improved Pharmacokinetic Profiles. *J. Med. Chem.* **1999**, *42*, 2087−2104.
(26) For an example where the mono fluorinated compound is superior to the difluorinated compound, see: Zhu, Y. P.; et al. Phenylcyclobutyl triazoles as selective inhibitors of 11β-hydroxysteroid dehydrogenase type I. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3412−3416.
(27) Coulthard, G.; Erb, W.; Aggarwal, V. K. Stereocontrolled organocatalytic synthesis of prostaglandin $PGF_{2\alpha}$ in seven steps. *Nature* **2012**, *489*, 278−281.
(28) Pelss, A.; Gandhamsetty, N.; Smith, J. R.; Mailhol, D.; Silvi, M.; Watson, A. J. A.; Perez-Powell, I.; Prévost, S.; Schützenmeister, N.; Moore, P. R.; Aggarwal, V. K. Re-optimization of the Organocatalyzed Double Aldol Domino Process to a Key Enal Intermediate and its Application to the Total Synthesis of Δ^{12}-Prostaglandin J_3. *Chem. - Eur. J.* **2018**, *24*, 9542−9545.
(29) Forster, A.; Fitremann, J.; Renaud, P. Preparation of an advanced intermediate for the synthesis of epi-thromboxanes. *Tetrahedron Lett.* **1998**, *39*, 3485−3488.
(30) Leonard, J.; Ouali, D.; Rahman, S. K. A short enantioselective route to corynanthe alkaloid precursors. *Tetrahedron Lett.* **1990**, *31*, 739−742.
(31) Scott, R. W.; Mazzetti, C.; Simpson, T. J.; Willis, C. L. γ-lactones from δ-lactones: total synthesis of the biosynthetic derailment product mupirocin H. *Chem. Commun.* **2012**, *48*, 2639−2641.
(32) Mckay, C.; Simpson, T. J.; Willis, C. L.; Forrest, A. K.; O'Hanlon, P. J. A versatile approach to the total synthesis of the pseudomonic acids. *Chem. Commun.* **2000**, 1109−1110.
(33) Yoshimitsu, T.; Yanagisawa, S.; Nagaoka, H. Asymmetric Synthesis of the Core Structure of (−)-CP-263,114. *Org. Lett.* **2000**, *2*, 3751−3754.
(34) McAtee, J. J.; Schinazi, R. F.; Liotta, D. C. A Completely Diastereoselective Electrophilic Fluorination of a Chiral, Noncarbohydrate Sugar Ring Precursor: Application to the Synthesis of Several Novel 2'-Fluoronucleosides. *J. Org. Chem.* **1998**, *63*, 2161−2167.
(35) Dupradeau, F.-Y.; Hakomori, S.-i.; Toyokuni, T. Electrophilic azidation of 2-deoxy-aldono-1,5-lactones: an alternative route to 2-azido-2-deoxy-aldopyranoses. *J. Chem. Soc., Chem. Commun.* **1995**, 221−222.
(36) The formation of the corresponding epoxide was unexpected since it involves displacement of fluoride but being a strained cyclic ether with the same stereochemistry it bore some similarity to TxA_2. It was therefore deprotected and tested for biological activity, but it (9,10-epoxy TxA_2) was essentially inactive.



Epoxide from Wittig reaction 9,10-Epoxy TxA_2

(37) Trost, B. M.; Wrobleski, S. T.; Chisholm, J. D.; Harrington, P. E.; Jung, M. Total Synthesis of (+)-Amphidinolide A. Assembly of the Fragments. *J. Am. Chem. Soc.* **2005**, *127*, 13589−13597.
(38) Trost, B. M.; Harrington, P. E.; Chisholm, J. D.; Wrobleski, S. T. Total Synthesis of (+)-Amphidinolide A. Structure Elucidation and Completion of the Synthesis. *J. Am. Chem. Soc.* **2005**, *127*, 13598−13610.
(39) Swarts, B. M.; Guo, Z. W. Chemical Synthesis of Glycosylphosphatidylinositol Anchors. *Adv. Carbohydr. Chem. Biochem.* **2012**, *67*, 137−219.
(40) Soliman, S. E.; Bennett, C. S. Reagent-Controlled Synthesis of the Branched Trisaccharide Fragment of the Antibiotic Saccharomicin B. *Org. Lett.* **2018**, *20*, 3413−3417.
(41) Brumsted, C. J.; Carpenter, E. L.; Indra, A. K.; Mahmud, T. Asymmetric Synthesis and Biological Activities of Pactamycin-Inspired Aminocyclopentitols. *Org. Lett.* **2018**, *20*, 397−400.
(42) Matsumori, N.; Umegawa, Y.; Oishi, T.; Murata, M. Bioactive fluorinated derivative of amphotericin B. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3565−3567.
(43) Suzuki, K.; Ohtake, A.; Ito, Y.; Kanie, O. Synthesis of a fluorescently tagged sialic acid analogue useful for live-cell imaging. *Chem. Commun.* **2012**, *48*, 9744−9746.
(44) Suzuki, K.; Daikoku, S.; Son, S.-H.; Ito, Y.; Kanie, O. Synthetic study of 3-fluorinated sialic acid derivatives. *Carbohydr. Res.* **2015**, *406*, 1−9.
(45) Bhagwat, S. S.; Hamann, P. R.; Still, W. C. Synthesis of Thromboxane A_2. *J. Am. Chem. Soc.* **1985**, *107*, 6372−6376.
(46) Mitsunobu, O. The Use of Diethyl Azodicarboxylate and Triphenylphosphine in Synthesis and Transformation of Natural Products. *Synthesis* **1981**, *1981*, 1−28.
(47) Isobe, T. 2-Chloro-1,3-dimethylimidazolinium Chloride. 3. Utility for Chlorination, Oxidation, Reduction, and Rearrangement Reactions. *J. Org. Chem.* **1999**, *64*, 5832−5835.
(48) Tanaka, T.; Huang, W. C.; Noguchi, M.; Kobayashi, A.; Shoda, S.-I. Direct synthesis of 1,6-anhydro sugars from unprotected glycopyranoses by using 2-chloro-1,3-dimethylimidazolinium chloride. *Tetrahedron Lett.* **2009**, *50*, 2154−2157.
(49) Koenigs, W.; Knorr, E. Ueber einige Derivate des Traubenzuckers und der Galactose. *Ber. Dtsch. Chem. Ges.* **1901**, *34*, 957−981.
(50) Fried reported a 10^8 difference of second-order rate constants for the hydrolysis of 10,10-F_2-TxA_2 model compound **3** at pH 1.27 (22 °C) with that of TxA_2 at pH 7.4 (37 °C). This translates to a 10^2 decrease in pseudo first-order rate constants (1.4×10^{-4} vs 2.2×10^{-2}) and half-lives of 86 min and 32 seconds, for the fluorinated model compound **3** (at pH 1.27) and TxA_2 (at pH 7.4), respectively.
(51) Szatylowicz, H.; Jezuita, A.; Siodła, T.; Varaksin, K. S.; Domanski, M. A.; Ejsmont, K.; Krygowski, T. M. Toward the Physical Interpretation of Inductive and Resonance Substituent Effects and Reexamination Based on Quantum Chemical Modeling. *ACS Omega* **2017**, *2*, 7163−7171.
(52) Withers, S. G.; Percival, M. D.; Street, I. P. The synthesis and hydrolysis of a series of deoxy- and deoxyfluoro-α-d- "glucopyranosyl" phosphates. *Carbohydr. Res.* **1989**, *187*, 43−66.
(53) Coleman, R. A.; Humphrey, P. P. A.; Kennedy, I.; Levy, G. P.; Lumley, P. Comparison of the actions of U-46619, a prostaglandin H_2-analogue, with those of prostaglandin H_2 and thromboxane A_2 on some isolated smooth muscle preparations. *Br. J. Pharmacol.* **1981**, *73*, 773−778.
(54) di Minno, G.; Bertelé, V.; Bianchi, L.; Barbieri, B.; Cerletti, C.; Dejana, E.; de Gaetano, G.; Silver, M. J. Effects of an Epoxymethano Stable Analogue of Prostaglandin Endoperoxides (U-46619) on Human Platelets. *Thromb. Haemostasis* **1981**, *45*, 103−106.
(55) Liel, N.; Mais, D. E.; Halushka, P. V. Binding of a thromboxane A_2/prostaglandin H_2 agonist [^{3}H]U46619 to washed human platelets. *Prostaglandins* **1987**, *33*, 789−797.
(56) Morinelli, T. A.; Niewiarowski, S.; Daniel, J. L.; Smith, J. B. Receptor-mediated effects of a PGH_2 analogue (U46619) on human platelets. *Am. J. Physiol.* **1987**, *253*, H1035−H1043.

https://dx.doi.org/10.1021/acscentsci.0c00310
ACS Cent. Sci. 2020, 6, 995−1000