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Total synthesis of diptoindonesin G and its analogues as selective modulators of estrogen receptors†

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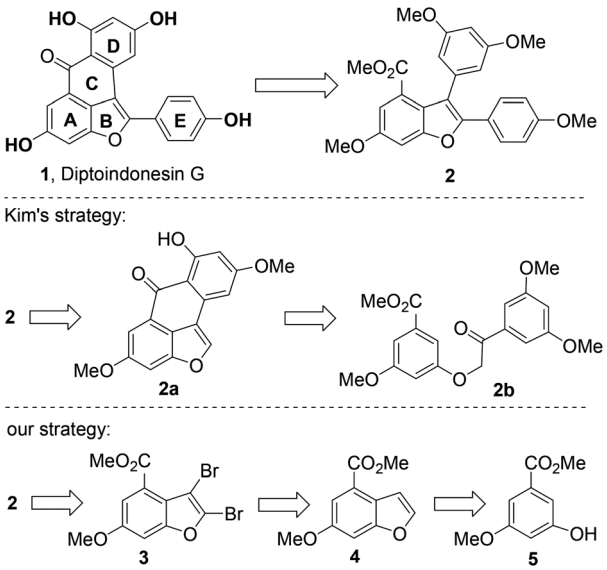
We have developed a versatile synthetic strategy for the synthesis of the natural product diptoindonesin G and its analogues as selective modulators of estrogen receptors. The strategy involves a regioselective dehydrative cyclization of arylacetals, a regioselective bromination of benzofurans, a sequential cross-coupling of bromo-benzofurans with aryl boronic acids, and a BBr_3-mediated tandem cyclization and demethylation. Preliminary biological studies uncovered the critical and dispensable phenolic hydroxyl groups in the natural product and also revealed unexpected selectivity for isoforms of estrogen receptor.

The natural product diptoindonesin (Dip) G **1** (Scheme 1) was isolated from tree barks of *Hopea mengarawan* in Indonesia together with nine other oligostilbenoids in 2009.[1] Around the same time, the same molecule was also isolated from *Hopea chinensis* stem barks in China.[2] Dip G has a rather unusual tetracyclic core with A–D rings bearing a ketone and three phenolic OH groups and an additional E-ring with one more phenolic OH group. Dip G showed anti-proliferation effect in murine leukemia P-388 cells[1] and immunosuppressant activity in a concanavalin A induced proliferation of mouse splenic lymphocytes (T cells) assay.[2] Recently, we reported that Dip G could regulate the stability of estrogen receptor α (ERα) and estrogen receptor β (ERβ), two members of the steroid nuclear receptor superfamily with opposing effect on cell proliferation.[3] ERα promotes cell proliferation, while ERβ has an anti-proliferative effect in breast cancer cells.[4] Interestingly, Dip G decreases the stability of the oncogenic ERα and increases the stability of ERβ, a tumor suppressor in breast cancer. Instead of directly interacting with ERs, Dip G was found to target the E3 ubiquitin ligase C-terminus of HSC70-interacting protein (CHIP), also known as STIP1 homology and U-Box containing protein 1 (STUB1).[3] By reciprocally regulating ER levels, natural product Dip G or its analogues may be developed as novel therapeutics for the treatment of breast cancers.



Scheme 1 Retrosynthetic analysis of Dip G.

The first and only total synthesis of Dip G was reported by Kim's group in 2010.[5] The key features of this synthesis include an elegant domino cyclization to construct both B and C rings of **2a** from diaryl ether **2b** and a Pd-catalyzed C–H arylation to install the E-ring in the penultimate step. For further biological studies, we are in need of a new synthetic strategy that is flexible for the practical synthesis of Dip G and its analogues as selective modulators of ERs. Ring C of **1** could be constructed by Friedel–Crafts acylation of a carboxylic acid

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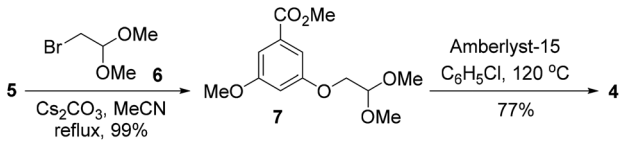
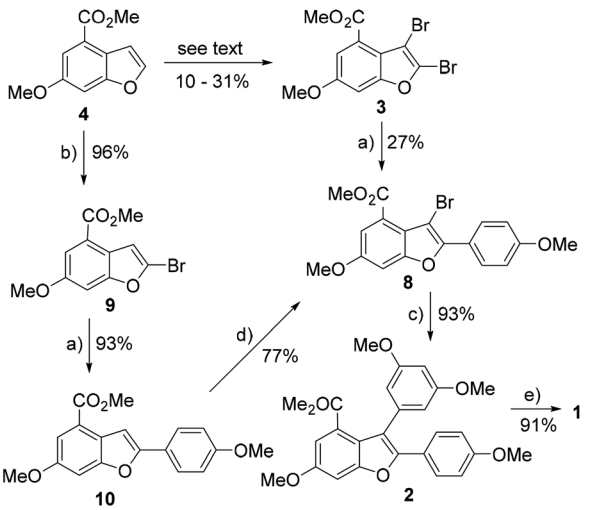
derivative of **2**. We envisioned that the permethylated intermediate **2** could be derived from dibromobenzofuran **3** efficiently by sequential cross-coupling with two aryl boronic acids. A sequence of alkylation, cyclization, and dibromination can convert resorcinol derivative **5** to dibromobenzofuran **3** *via* benzofuran **4**. The modularity of this strategy will allow us to access various Dip G analogues by simply changing the aryl boronic acid reagents at the late stage.

Mono-protected resorcinol derivative **5** is commercially available. It can also be prepared conveniently from methyl 3,5-dihydroxybenzoate. The benzofuran core **4** was synthesized efficiently from **5** by the sequence of alkylation with bromodimethylacetal and cyclodehydration (Scheme 2) using Amberlyst-15.[6] The cyclization occurred regioselectively, which is consistent with similar reactions reported previously.[6]

Although there are a number of reports on dibromination of unsubstituted benzofurans,[7] we were not able to prepare **3** from **4** directly in reliable yields after screening various bromination reagents, solvents, bases, and temperature (Scheme 3). The carboxylate ester substituent on the 4-position appeared to interfere with the second bromination. The cross-coupling occurred selectively on the 2-position of benzofuran **3**. However, the yield of product **8** was low. Although we were able to do the sequential Pd-catalyzed cross-coupling reaction for dibromobenzofuran **3**,[8] the yield was low for the first step.



Scheme 2 Synthesis of the benzofuran core of Dip G.



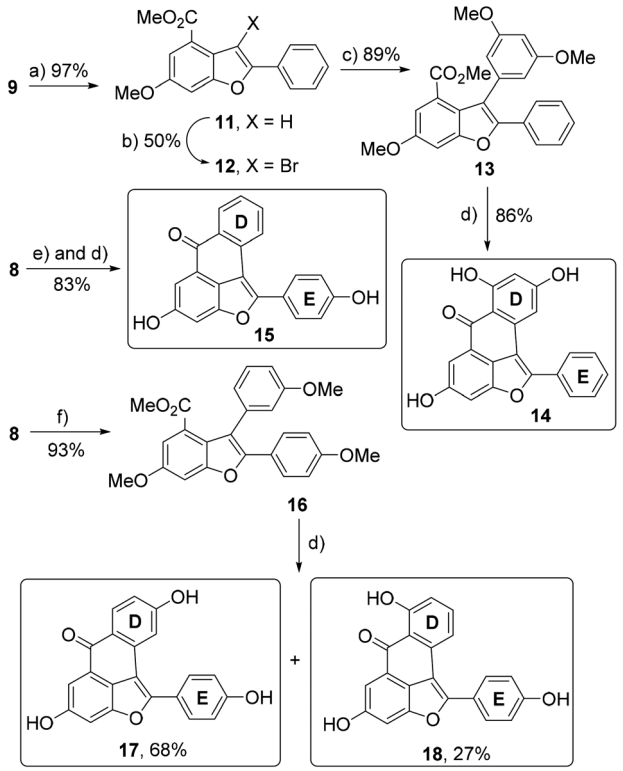
Scheme 3 Synthesis of Dip G **1** by sequential bromination of benzofurans and Pd-catalyzed cross-couplings.

During the course of optimizing the dibromination of benzofuran **4**, we found that mono-bromination occurred quickly. The second bromination was very slow, and decomposition of brominated products started to occur at high temperature. 2-Bromobenzofuran **9** was isolated in high yield as the only isomer when we used dichloroethane as the solvent and DMF as the catalyst. The direct bromination of benzofurans generally occurred on the more reactive 3-position.[9] 2-Bromobenzofurans were usually prepared by lithiation and quenching with electrophilic bromination reagents.[10] We hypothesized that the carboxylate ester substituent on the 4-position deactivated the 3-position for bromination and this led to the formation of 2-bromobenzofuran only. Pd-catalyzed cross-coupling of 2-bromobenzofuran **9** with 4-methoxyphenyl boronic acid occurred smoothly to afford product **10**. 3-Bromobenzofuran **8** could be obtained in high yield from **10** after stopping the reaction at around 10 min. Longer reaction time led to lower yield of product **8**. The second cross-coupling with 3,5-dimethoxyphenyl boronic acid occurred at higher temperature to yield penultimate intermediate **2**. Natural product Dip G **1** was prepared from **2** by BBr_3 mediated tandem cyclization and demethylation following literature procedure.[5] Up to 0.4 g of Dip G precursor **2** was prepared using this synthetic route.

To examine the effect of the four phenolic OH groups on biological activity, we next prepared Dip G analogues with just two or three phenolic OH groups as shown in Scheme 4. Simply replacing the *para*-methoxyphenyl boronic acid by phenyl boronic acid, analogue **14** lacking the phenolic OH group in the E-ring of Dip G was prepared from bromobenzofuran **9** in four steps according to the sequence outlined in Scheme 3 for the preparation of **1** from **9**. The bromination of 2-phenyl substituted benzofuran **11** proved to be more difficult than the bromination of substrates **4** or **10**. Only trace amount of 3-bromobenzofuran **12** was obtained under the conditions shown in Scheme 3 for substrates **4** or **10**. The yield could be improved to 50% by using DMF as the co-solvent.

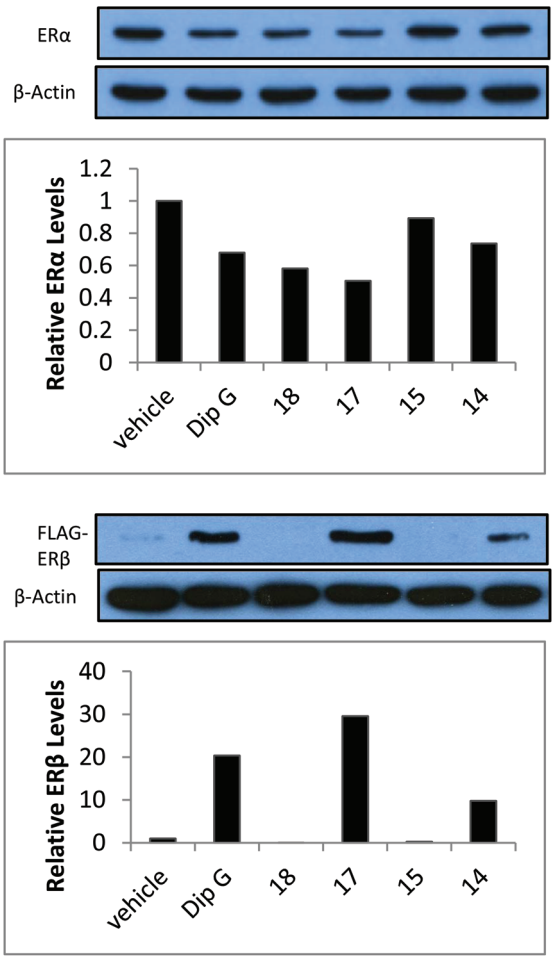
Analogue **15** that missed both phenolic OH groups in the D-ring was synthesized from intermediate **8** in just two steps. We were pleased to find that the Friedel–Crafts cyclization still worked smoothly for the formation of **15** after replacing the electron-rich dimethoxyphenyl group in Dip G **1** by a simple phenyl group in **15**. Finally, we prepared compound **16** with just one methoxy group in the D-ring from intermediate **8**. Ideally, we want to access both products **17** and **18** to investigate the effect of each one of the two phenolic OH groups in the D-ring of Dip G. As expected, both products were formed and the acylation occurred preferentially on the less-hindered position of the D-ring.

The biological activity of the above four analogues were then compared with the parent compound Dip G following our previous protocol (Scheme 5).[3] Cells were treated with each compound at 10 μM concentration in order to evaluate how well each modified the stability of ERα and ERβ. In MCF7



a) $Pd(PPh_3)_4$ (5 mol%), K_2CO_3, $PhB(OH)_2$, DMF, 75 °C;
b) NBS, $ClCH_2CH_2Cl$/DMF (5:1), 80 °C;
c) $Pd(PPh_3)_4$ (5 mol%), K_2CO_3, 3,5-$(MeO)_2C_6H_3B(OH)_2$, DMF, 110 °C;
d) excess BBr_3, CH_2Cl_2, -78 °C;
e) $Pd(PPh_3)_4$ (5 mol%), K_2CO_3, $PhB(OH)_2$, DMF, 110 °C;
f) $Pd(PPh_3)_4$ (5 mol%), K_2CO_3, 3-$MeOC_6H_4B(OH)_2$, DMF, 100 °C

Scheme 4 Synthesis of Dip G analogues.



Scheme 5 Activities of Dip G analogues to modify ER stability.

cells, Dip G and all four analogs showed decreased ERα stability with the largest change occurring with analogues **17** followed by **18** and Dip G. In Hs578T-ERβLuc Dox-inducible lines, ERβ was strongly stabilized by Dip G and compound **17**. ERβ was moderately stabilized by compound **14**. Surprisingly, compounds **15** and **18** both destabilized ERβ, suggesting that the hydroxyl group para to the ketone in the D-ring of Dip G was critical for stabilizing ERβ. Compound **15** is interesting as it does not significantly modulate the stability of ERα and this compound can be a useful chemical probe for the study of the function of ERβ. The hydroxyl group *ortho* to the ketone in the D-ring of Dip G is dispensable as the activity of compound **17** was slightly better than Dip G for destabilizing ERα and stabilizing ERβ. This is very critical for further improving the pharmacological properties of Dip G analogues and accessing different chemical probes.

In summary, we have developed a practical and flexible synthetic strategy for the preparation of Dip G. This new strategy allows us to access Dip G analogues with just two or three of the four phenolic hydroxyl groups systematically. Preliminary biological evaluation of these new analogues revealed that not all four phenolic hydroxyl groups were required for activity. Selective modulators for both isoforms of ERα and ERβ were discovered in this study. This paved the way for further structure activity relationship studies and the development of new chemical probes for ERs, which would be reported in due course.

Acknowledgements

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