EurJOC
European Journal of Organic Chemistry

A Journal of
ChemPubSoc Europe

Accepted Article

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To be cited as: *Eur. J. Org. Chem.* 10.1002/ejoc.201701811

Link to VoR: http://dx.doi.org/10.1002/ejoc.201701811

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First total syntheses of Amorfrutin C and *pseudo*-Amorfrutin A

Qi Miao[a], Yunzhi Li[a], Jinyi Xu[a], Aijun Lin[a], Genzoh Tanabe[b], Osamu Muraoka[b], Xiaoming Wu[a]* and Weijia Xie[a]*

Abstract: Syntheses of Amorfrutin C as a potent antitumor natural product as well as *pseudo*-Amorfrutin A were accomplished. Protected 2, 4-dihydroxybenzoic acid derivative (**5**) was used as a common synthetic skeleton. The introduction of prenyl moiety to **5** could be achieved through bromination reaction and a subsequent CuCN-meditated alkylation reaction. Interestingly, a selective NBS-mediated monobromination at 6-position provided us *pseudo*-Amorfrutin A whereas DBDMH triggered bromination at both 6,8-positions lead us to naturally occurring Amorfrutin C.

Introduction

Amorfrutin family bearing a characteristic planar 2-hydroxybenzoic acid core with prenyl, benzyl, or alkyl residues and a methoxy or hydroxy group attached at C-4 position usually exhibit diversified bioactivities.[1] Amorfrutin A was first extracted from *Glycyrrhiza acanthocarpa* by Ghisalberti *et al*.[2] Biological studies revealed that Amorfrutin A possesses potent antimicrobial,[3] anticancer,[4] anti-inflammatory[5] and anti-diabetes[6] activities. Mitscher *et al* extracted Amorfrutin A and Amorfrutin B from *Amorpha fruticosa*.[3] In the biological investigation, Amorfrutin B was found to have potent peroxisome proliferator-activated receptor (PPAR-γ) activation ability [7] and moderate cytotoxic activity.[6] Amorfrutin 2, first isolated from *Glycyrrhiza acanthocarpa* by Ghisalberti *et al*,[2] was found to have potent PPAR-γ activation ability. In 2016, Sauer *et al* extracted a known PPAR-γ activator Amorfrutin B and a structurally related new natural product Amorfrutin C from roots of *Glycyrrhiza foetida* (22 mg in 1 kg of air-dried and powdered roots).[6] Despite a large level of structural similarity between Amorfrutin B and Amorfrutin C, the later one exhibited low efficacy for PPAR-γ activation, but possess striking inhibition against human cancer cells proliferation. For example, Amorfrutin C could inhibit HT-29, T84, PC-3 and MCF-7 at the level of 10 μM, stronger than cisplatin and comparable with fluorouracil (5-FU). Further mechanism studies revealed that Amorfrutin C could induce poly ADP-ribose polymerase (PARP) cleavage, phosphatidylserine externalization, and formation of reactive oxygen species.[6] Most recently, a new compound of amorfrutin family, Amorfrutin D, was isolated from *Amorpha fruticosa* by Muharini *et al*, although biological evaluation of this compound has not been reported yet.[8] Based on the existing natural Amorfrutin derivatives, it is of high possibility that the substitution of alkyl groups on phenyl ring may have deep effects not only on biological activity potency but also on different bioactive profiles of these natural products.

Total syntheses and biological evaluation of Amorfrutin A[9] and Amorfrutin B [7, 10] were extensive investigated, while few attention was attached on the synthesis of Amorfrutin C. Herein we first report an efficient gram-scale total synthesis of Amorfrutin C, during which process, its 5-monoprenylated analog (*pseudo*-Amorfrutin A) was also obtained. To the best of our knowledge, derivative with mono-alkylation at C-5 position has not been identified within Amorfrutin family. It is believed that design and synthesis of *pseudo*-Amorfrutin A would play a positive role for both structural diversification and structure-activity relationship (SAR) studies on this group of natural products.

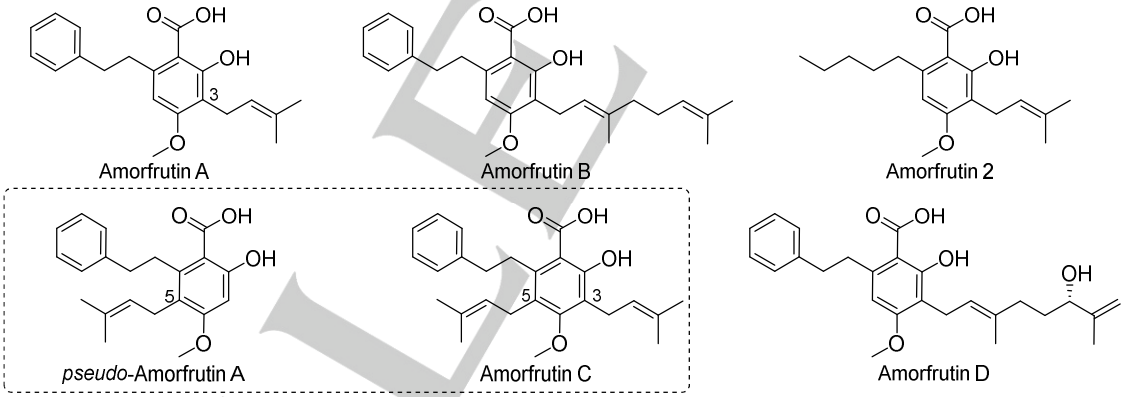

Figure 1. Structures of naturally occurring Amorfrutin family and synthetic pseudo-Amorfrutin A.

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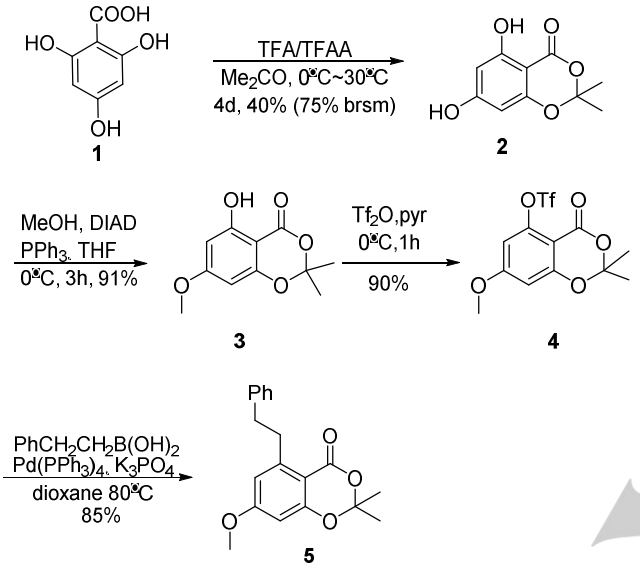
Results and Discussion

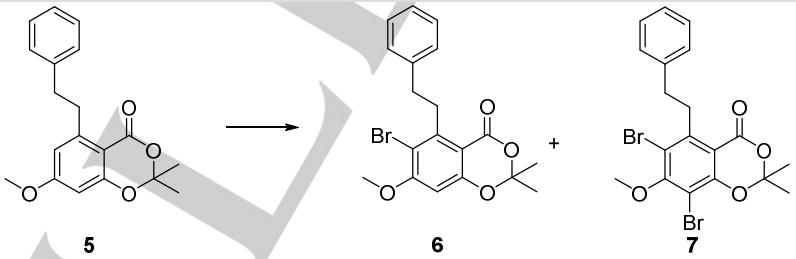
Thus, commercially available 2,4,6-trihydroxybenzoic acid (**1**) was first subjected to standard acetonization reaction to give acetonide(**2**) in 75% yield. The intramolecular hydrogen bond between C-5 hydroxyl with the adjacent carbonyl group of **2** facilitated selective methylation of the C-7 hydroxyl[11] to give phenol (**3**) with the yield of 91%. Triflation reaction of **3** with trifluoromethanesulfonic anhydride and pyridine provided target triflate (**4**) in good yield. Replacement of trifluoromethanesulfonic ester of triflate (**4**) by phenethyl group was first reported by Weidner *et al* through Sonogashira coupling and continuous catalytic hydrogenation.[9a] In the present study, it was found that direct Suzuki coupling reaction was also effective for the alkylation. Treatment of **4** with commercially available phenethylboronic acid and catalytic amount of $Pd(PPh_3)_4$ provided acetonide (**5**) in satisfactory yield (Scheme 1).



Scheme 1. Synthetic approach towards acetonide (**5**); TFA = trifluoroacetic acid; TFAA = trifluoroacetic anhydride; brsm = yield based on recovering starting material; DIAD = diisopropyl azodiformate.

With **5** in hand, we then explored the bromination condition. According to literature,[12] N-bromosuccinimide (NBS) was frequently used as a bromination reagent on phenyl ring structure. However, under the reported reacting condition, only monobromide (**6**) was identified in poor yield (Table 1, entry 1). When the reacting solution was refluxed for 8 hours, only trace amount of target dibromide (**7**) was identified (Table 1, entry 2). When reacting solvent was changed to dichloromethane (DCM), **5** could be converted to monobromide **6** as a single product in 82% yield (Table 1, entry 3). It was reported that addition of methanol (MeOH) into the reacting system could facilitate the bromination process.[13] Thus, in a mixed DCM and MeOH solution, the consumption of **5** was dramatically accelerated, however, mono-bromide (**6**) was obtained in 81% after 4 hours as the exclusive product (Table 1, entry 4). The bromination reagent was then changed to dibromohydantoin (DBDMH).[14] To our delight, after stirring at room temperature for 24h, the desired dibrominated compound (**7**) was obtained in 33% yield (Table 1, entry 5). It was reported that the introduction of pyridinium salt could further improve the bromination efficacy.[15] When pyridinium 4-toluenesulfonate (PPTS) was added, isolation yield of dibromide **7** was improved to 86% (Table 1, entry 6).

Table 1. Bromination reaction condition of acetonide (**5**)

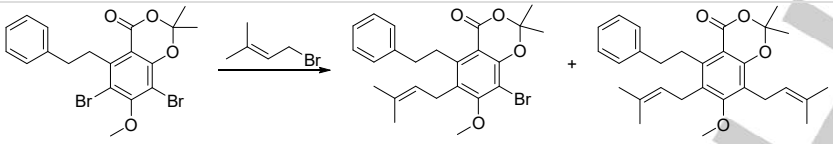


5 **6** **7**

	Bromination reagent	Solvent	Temp.(°C)	Time(h)	Yield[d]	
					6	**7**
1	NBS[a]	THF	0 to 25	18	15%	0
2	NBS[a]	THF	0 to reflux	8	10%	Trace
3	NBS[a]	DCM	0 to reflux	48	82%	0
4	NBS[a]	DCM+MeOH[c]	0 to reflux	4	81%	0
5	DBDMH[a]	DCM	0 to 25	24	35%	33%
6	DBDMH+p-TSA·py[b]	DCM	0 to 25	18	0	86%

[a] NBS = N-bromosuccinimide, 3.0 eq. [b] DBDMH= dibromohydantoin, 4 eq; p-TSA·py = pyridinium 4-toluenesulfonate, 6 eq. [c] DCM = dichloromethane; MeOH = methanol; DCM:MeOH = 2:1 (v:v). [d] isolated yield.

Table 2. Alkylation reaction condition of dibromide (**7**)



7 **8** **9**

Entry	Copper (I) source	Solvent	Base	Temp.	Yield (%)[f]	
					8	**9**
1	CuCN·2LiCl[a]	THF	n-BuLi[d]	-78 to r.t.	10	N.D.
2	CuCN·2LiCl[a]	THF	n-BuLi[e]	-78 to r.t	48	N.D.
3	CuCN·2LiCl[b]	THF	n-BuLi[e]	-78 to r.t	42	N.D.
4	CuCN·2LiCl[a]	THF+Toluene[c]	n-BuLi[e]	-78 to r.t.	61	N.D.
5	CuCN·2LiCl[a]	THF+Toluene[c]	n-BuLi[e]	-100 to r.t.	72	N.D.

[a] 30 mol-%. [b] 2 eq. [c] THF: Toluene = 2:1. [d] 2.5M, 1.05 eq. [e] 1.6M, 1.05 eq. [f] isolated yield.

Alkylation of the dibromide (**7**) was then systematically investigated. Different metal-catalyzed coupling reactions such as Negishi coupling and Suzuki coupling were attempted which were proved ineffective for our system. It was reported that copper (I) reagent could be used for Sp^2-Sp^3 coupling reaction.[16] When CuCN·2LiCl (30% mol) was applied, only the mono-prenylated product **(8**) can be detected in poor yield (Table 2, entry 1). When *n*-butyllithium (*n*-BuLi) at lower concentration was used, the yield of **8** was remarkably increased, however, no formation of diprenylated compound (**9**) was detected (Table 2, entry 2). Increasing the loading of CuCN·2LiCl was not effective either (Table 2, Entry 3). It was reported that a mixture of non-polar solvent might be crucial for a clean conversion of alkylation reaction.[17] The addition of toluene into the reacting system thus further improved the yield of **8** to 61% (Table 2, entry 4). When the lithium-bromide exchange process was performed under an even lower temperature (-100ºC), **8** was acquired in a desirable yield (Table 2, entry 5).

Our next attempt was to apply the same prenylation reaction to the isolated mono-prenylated compound (**8**) which provided us the target intermediate (**9**) with the yield of only 9% (Table 3, entry 1). Decreasing reacting temperature could improve the yield of **9** to 30% which was still unsatisfactory (Table 3, entry 2). The electric property and the steric hindrance of C-8 between two oxygen may cause instability of corresponding organolithium or organocopper intermediates which could directly hamper the alkylation process.[18] As such, we speculated that the introduction of organometallic stabilizer might be beneficial for reaction yield improvement. Two different kind of common organometallic stabilizers tetramethylethylenediamine (TMEDA) and hexamethylphosphorictriamide (HMPA) were used (Table 3, entry 3 & 4). To our delight, HMPA was found effective and **9** could be achieved in a moderate yield (Table 3, entry 4). Finally, base catalyzed deprotection was performed under regular

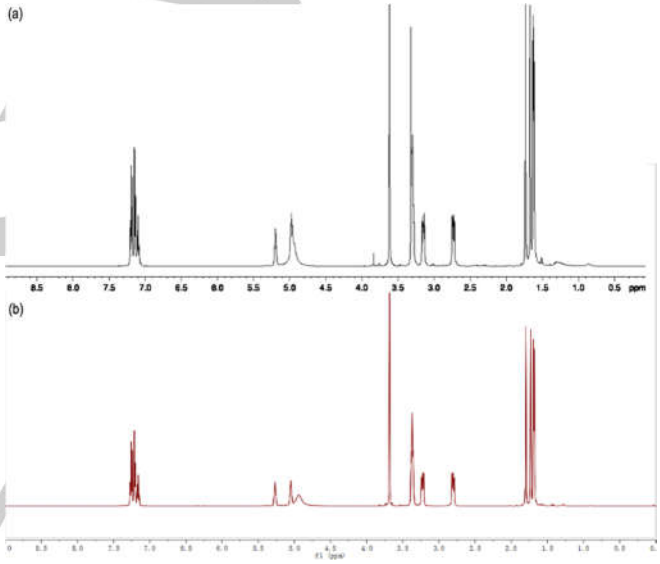
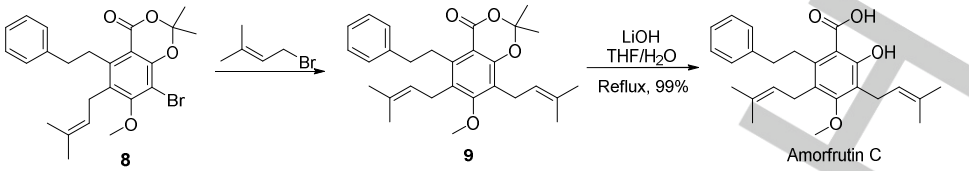


Figure 2. ^{1}H-NMR spectra of natural (a) and synthetic (b) Amorfrutin C

condition to provide Amorfrutin C quantitatively. The NMR spectroscopic property of the synthesized Amorfrutin C was in exact accord with those of previously reported (Figure 2). [6]

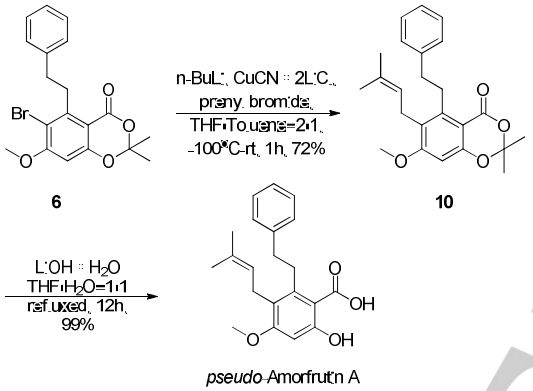
It is interesting to note that all Amorfrutin derivatives identified by far featured a 3-alkylated substitution on phenyl ring. Compared with 3-monoalkylated Amorfrutin A, the additional attachment of prenyl moiety at 5-position provided Amorfrutin C with totally different biological activities. We believe that the construction of 5-mono-alkylated Amorfrutin analogs would offer us more opportunities for structural and bioactive diversification of this series of natural products. Thus, we further introduced prenyl group to mono-brominated compound (**6**) by applying similar method discussed above to give *pseudo*-Amorfrutin A with the total yield of 29.3% from **1**.

Table 3. Alkylation of mono-alkylation compound (**9**) and synthesis of Amorfrutin C



Entry	Copper reagent[a]	Solvent[b]	Base[c]	Additive[d]	Temp.	Yield[e]
1	CuCN·2LiCl	THF+Toluene	n-BuLi	-	-78 to r.t.	9%
2	CuCN·2LiCl	THF+Toluene	n-BuLi	-	-100 to r.t.	30%
3	CuCN·2LiCl	THF+Toluene	n-BuLi	TMEDA	-100 to r.t.	30%
4	CuCN·2LiCl	THF+Toluene	n-BuLi	HMPA	-100 to r.t.	50%

[a] 30 mol-%. [b] THF:Toluene = 2:1 (v:v). [c] 1.6 M. [d] 5 eq. [e] isolated yield.



Scheme 2. Synthetic approach towards *pseudo*-Amorfrutin A

Conclusions

In summary, the first total synthesis of Amorfrutin C was accomplished in 8 steps from commercially available 2,4,6-trihydroxybenzoic acid with an overall yield of 16%. When different bromination reagent was applied, *pseudo*-Amorfrutin A could also be obtained with the total yield of 29.3%. NBS was found as an effective reagent for selective C-6 bromination, whereas DBDMH could provide dibromide intermediate. As for the synthesis of Amorfrutin C, a two-step continuous alkylation of dibromide was performed and the addition of organometallic stabilizer HMPA was found imperative for the introduction of second prenyl group. The present study is important for further design and synthesis of Amorfrutin family derivatives with alkyl groups substituted at different phenyl ring position in order to identify ideal drug candidates with higher potency, target selectivity and lower adverse effects.

Experimental Section

General: All reactions were carried out in oven-dried glassware, using commercially supplied solvents and reagents unless otherwise stated. THF and toluene were redistilled from Na-Ph_2CO.Hexamethylphosphorictriamide (HMPA) was redistilled from CaO and Na under reduced pressure. Prenyl bromide was dried with Mg_2SO_4 overnight, and redistilled at 2 mm pressure. The middle fraction (*ca* 55 °C) was collected. All reaction have been monitored by thin layer chromatography (TLC). TLC was performed on silica gel 60 F254 plates (glass plates). Column chromatography was performed using silica gel (200-300 mesh) packed in glass columns. Yields refer to purified, dried and spectroscopically pure compounds. NMR spectra were recorded with 300 MHz (^{1}H: 300 MHz, ^{13}C: 75 MHz), or 500 MHz (^{1}H:500 MHz, ^{13}C: 125 MHz) spectrometers.Chemical shifts (δ-values) are reported in ppm, spectra were calibrated related to solvents' residual proton chemical shifts ($CDCl_3$, δ = 7.26), (Methanol-d_4, δ = 3.30) or (Acetone-d_6. δ = 2.05) and solvents' residual carbon chemical shifts ($CDCl_3$, δ = 77.0) , (MeOH-d_4, δ = 49.0) and (Acetone-d_6. δ = 29.84), multiplicity is reported as follows: s = singlet, brs = broad singlet, d = doublet, dd = doublet of doublet, t = triplet, q =quartet, m = multiplet or unresolved and coupling constant *J* in Hz. High resolution electrospray ionization mass spectroscopy (HRMS) were obtained by electrospray ionization using a Q-TOF mass spectrometer in positive ion mode ($M + H^+$ or $M + Na^+$) as indicated. Melting points are uncorrected.

Experimental Procedure:

7-methoxy-2,2-dimethyl-5-phenethyl-*4H*-1,3-benzodioxin-4-one (**5**)**:** A mixture of triflate[9a](**4**) (1.5 g, 4.2 mmol), phenethylboronic acid (1.3 g, 8.4 mmol), K_3PO_4 (2.7 g, 12.7 mmol) and $Pd(PPh_3)_4$ (484 mg, 10% mmol) were added to a rubber-capped schlenk flask and the resulted mixture was protected with argon. 1,4-dioxane (20 mL) was then added to the Schlenk vessel. The resulting mixture was stirred at 80 °C overnight. The reaction mixture was filtered through diatomite and the filtrate was concentrated under reduced pressure. The crude product was subjected to silica gel column chromatography (hexane/EtOAc = 100:1 ~ 90:1) to afford **5** (1.1 g, 85%) as a light yellow solid. m.p. 137-138 °C. ^{1}H NMR (300 MHz, Acetone-d_6): δ = 7.28 - 7.27 (m, 4H), 7.21 - 7.03 (m, 1H), 6.58 (d, *J* = 2.4 Hz, 1H), 6.43 (d, *J* = 2.4 Hz, 1H), 3.84 (s, 3H),

3.35-3.30 (m, 2H), 2.97-2.69 (m, 2H), 1.66 (s, 6H) ppm. The NMR data is completely in accordance with previous literature[9a].

6-bromo-7-methoxy-2,2-dimethyl-5-phenethyl-*4H*-1,3-benzodioxin-4-one (**6**)**:** To the solution of **5** (31.2 mg, 0.1 mmol) in DCM (1.5 mL) and MeOH (0.75 mL) was added NBS (53.4 mg, 0.3 mmol), allowed to stir at r.t. for 3.5 h. After the complete conversion of starting material (monitored by TLC), the mixture was diluted with DCM, washed with aqueous $Na_2S_2O_3$, water and brine. The organic layer was then dried by anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was subjected to flash chromatography (hexane/EtOAc = 75:1) to afford **6** (31.5 mg, 80.6%) as a light yellow amorphous solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.41 – 7.17 (m, 5H), 6.40 (s, 1H), 3.93 (s, 3H), 3.66 – 3.61 (m, 2H), 2.89 – 2.83 (m, 2H), 1.68 (s, 6H) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 161.4, 159.4, 158.2, 147.2, 141.9, 128.9, 128.4, 126.1, 110.2, 106.3, 105.1, 98.8, 56.8, 36.3, 35.1, 25.7. HRMS (ESI): calcd for $C_{19}H_{19}{}^{35}BrO_4Na^+$ [M + Na]$^+$: 413.0359, found: 413.0359.

6,8-dibromo-7-methoxy-2,2-dimethyl-5-phenethyl-*4H*-1,3-benzodioxin-4-one (**7**)**:** To the solution of **5** (1.7 g, 5.4 mmol) and pyridinium 4-toluenesulfonate (8.1 g, 32.1 mmol) in dichloromethane (80 mL) was added 1,3-dibromo-5,5-dimethylhydantoin (6.1g, 21.4 mmol). The mixture was stirred at r.t. in dark overnight. After the complete conversion of the starting material, the mixture was diluted with DCM, washed with aqueous $Na_2S_2O_3$, water and brine. The organic layer was then dried by anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was subjected to flash chromatography (hexane/EtOAc = 200:1) to afford **7** (2.2 g, 86%) as a light yellow amorphous solid.^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.39 – 7.18 (m, 5H), 3.96 (s, 3H), 3.63 – 3.58 (m, 2H), 2.90 – 2.84 (m, 2H), 1.72 (s, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 159.9, 158.6, 154.5, 146.2, 141.4, 128.7, 128.5, 126.2, 116.7, 110.8, 106.0, 105.6, 60.8, 36.4, 34.9, 25.7 ppm. HRMS (ESI): calcd for $C_{19}H_{18}{}^{35}Br^{37}BrO_4Na^+$ [M + Na]$^+$: 492.9444, found: 492.9443.

8-bromo-7-methoxy-2,2-dimethyl-6-(3-methylbut-2-en-1-yl)-5-phenethyl-*4H*-1,3-benzodioxin-4-one (**8**)**: 7** (1.8 g, 3.8 mmol) was dissolved in distilled toluene (18 mL) and THF (36 mL) and cooled to -100 °C. n-BuLi (1.6 M, 4 mmol, 2.5 mL) was then added to this solution and the mixture was maintained at -100 °C for 30 min. Prepared CuCN•2LiCl (1.17 mL, 1.17 mmol)[19] and freshly distilled prenyl bromide (1.17 mL, 9.4 mmol) was subsequently added. The resulted solution was stirred at r.t. After 1 h, the reaction mixture was added a mixture of NH_3•H_2O and aqueous NaCl (1:1 v/v) and extracted with EtOAc (4 x). The organic layer was washed with water and brine, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude was then subjected to flash chromatography (hexane/EtOAc = 500:1) to afford **8** (1.27 g. 72%) as a light yellow solid. M.p.: 210-211 °C ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.34 – 7.10 (m, 5H), 5.01 (t, *J* = 6.9 Hz, 1H), 3.80 (s, 3H), 3.52 – 3.46 (m, 2H), 3.27 (d, *J* = 7.0 Hz, 2H), 2.82 – 2.76 (m, 2H), 1.70 (s, 3H), 1.61 (s, 3H), 1.59 (s, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 160.7, 159.6, 155.7, 144.6, 142.0, 132.7, 128.8, 128.5, 126.1, 124.0, 121.5, 116.3, 110.4, 105.1, 61.4, 36.5, 35.2, 25.8, 25.7, 23.7, 18.1 ppm. HRMS (ESI): calcd for $C_{24}H_{27}{}^{35}BrO_4Na^+$[M + Na]$^+$: 481.0985, found: 481.0985.

7-methoxy-2,2-dimethyl-6,8-bis(3-methylbut-2-en-1-yl)-5-phenethyl-*4H*-1,3-benzodioxin-4-one (**9**)**:8** (2.4 g, 5 mmol) was dissolved in distilled toluene (24 mL) and THF (48 mL) and cooled to -100°C. n-BuLi (1.6 M, 5.2 mmol, 3.3 mL) was added to this solution and the mixture was maintained at -100 °C for 25 min. Prepared CuCN•2LiCl (1.5 mL, 1.5 mmol), distilled HMPA (4.5 mL, 25 mmol) and freshly distilled prenyl bromide (1.56 mL,12.5 mmol) were subsequently added. The resulted solution was stirred at r.t. After 1 h, the reaction mixture was added a mixture of NH_3•H_2O and aqueous NaCl (1:1 v/v) and extracted with EtOAc (4 x). The organic layer was washed with water and brine, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude was then subjected to flash chromatography (hexane/EtOAc = 650:1) to afford **9** (1.12 g. 50%) as a light yellow solid. M.p. 219-220 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.35-7.26 (m, 4H), 7.21-7.19 (m, 1H), 5.13 (t, *J* = 6.4 Hz, 1H), 5.06 (m, 1H), 3.75 (s, 3H), 3.39 – 3.30 (m, 6H), 2.83 – 2.78 (m, 2H), 1.78 (s, 3H), 1.75 (s, 3H), 1.70 (s, 6H), 1.68 (s, 6H) ppm.^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.6, 160.6, 155.1, 144.6, 142.5, 131.9, 129.8, 128.7, 128.4, 126.0, 123.8, 122.4, 121.9, 109.2, 104.6, 37.1, 32.6, 25.8, 25.8, 25.3, 23.3, 18.2, 18.0 ppm. HRMS (ESI): calcd for $C_{29}H_{36}O_4Na^+$ [M + Na]$^+$: 471.2506, found: 471.2501.

2-hydroxy-4-methoxy-3,5-bis(3-methylbut-2-en-1-yl)-6-phenethylbenzoic acid (**Amorfrutin C**)**:** To the solution of **9** (1.12 g, 2.5 mmol) in THF (10 mL) and H_2O (10 mL) was added LiOH·H_2O (420 mg ,40 mmol). Then the mixture was heated to reflux overnight. The reaction mixture was then cooled to r.t. and diluted with EtOAc. 1M HCl was added to acidified the solution to slightly acid (pH = 2 ~ 3). The organic layer was separated and the water layer was extracted with EtOAc (3 x). Then the organic layer was mixed and washed with water and brine, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude was then subjected to flash chromatography (hexane/EtOAc = 10:1 ~ 5:1) to afford **Amorfrutin C** (1.01 g, 99%) as a white solid. M.p. 220-222 °C. ^{1}H NMR (500 MHz, methanol-d_4): δ = 7.23 (t, *J* = 7.4 Hz, 2H), 7.19 – 7.17 (m, 2H), 7.13 (t, *J* = 7.2 Hz, 1H), 5.24 (t, *J* = 6.6 Hz, 1H), 5.02 (m, 1H), 3.66 (s, 3H), 3.36 - 3.33 (m, 4H), 3.25 – 2.98 (m, 2H), 2.78 – 2.76 (m, 2H), 1.77 (s, 3H), 1.70 (s, 3H), 1.67 (s, 3H), 1.65 (s, 3H) ppm.^{13}C NMR (126 MHz, methanol-d_4): δ = 174.9, 162.6, 161.8, 143.7, 143.5, 132.0, 131.9, 129.2, 129.2, 126.9, 126.8, 125.7, 124.3, 122.0, 110.7, 61.9, 38.7, 34.3, 26.3, 25.9, 25.8, 24.3, 18.2, 18.0 ppm. HRMS (ESI): calcd for $C_{26}H_{32}O_4Na^+$[M + Na]$^+$: 431.2193, found: 431.2190.

7-methoxy-2,2-dimethyl-6-(3-methylbut-2-en-1-yl)-5-phenethyl-*4H*-1,3-benzodioxin-4-one (**10**)**: 6** (98 mg, 0.25 mmol) was dissolved in distilled toluene (1.2 mL) and THF (2.5 mL) and cooled to -100 °C. n-BuLi (1.6 M, 0.26 mmol, 164 µl) was added to this solution at -100 °C and the resulted reaction mixture was stirred at -100°C for 30min. Prepared CuCN•2LiCl (78 µL, 0.075 mmol) and freshly distilled prenyl bromide (78 µL, 0.625 mmol) was subsequently added. The resulted solution was stirred at r.t. After 1 h, the reaction mixture was added a mixture of NH_3•H_2O and aqueous NaCl (1:1 v/v) and extracted with EtOAc (4 x). The combine organic layer was washed with water and brine, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude was then subjected to flash chromatography (hexane/EtOAc = 500:1) to afford **10** (68 mg.

72%) as an off-white solid. M.p. 205-206 °C ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.26 – 7.17 (m, 5H), 6.31 (s, 1H), 5.10 (m, 1H), 3.78 (s, 3H), 3.34 – 3.23 (m, 4H), 2.92 – 2.87 (m, 2H), 1.75 (s, 3H), 1.67 (s, 9H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.0, 160.7, 155.7, 146.2, 142.0, 131.8, 128.9, 128.3, 125.9, 121.8, 116.2, 108.2, 105.4, 104.7, 55.7, 37.5, 37.4, 25.9, 25.8, 21.9, 17.8 ppm. HRMS (ESI): calcd for $C_{24}H_{28}O_4Na^+$ [M + Na]$^+$: 403.1880, found: 403.1878.

6-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)-2-phenethylbenzoic acid (***pseudo*-Amorfrutin A**)**:** To the solution of **10** (95 mg, 0.25 mmol) in THF (1 mL) and H_2O (1 mL) was added LiOH·H_2O (42 mg,1 mmol). The mixture was refluxed for 12 hours. The reaction mixture was cooled down to r.t. and diluted with EtOAc. 1M HCl was added to acidify the solution to slightly acid (pH = 2 ~ 3). The organic layer was separated and the water layer was extracted with EtOAc (3 x). The combined organic layer was washed with water and brine, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude was then subjected to flash chromatography (hexane/EtOAc = 10:1 ~ 5:1) to afford ***pseudo*-Amorfrutin A** (84 mg, 99%) as white solid. m.p. 210-211 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ = 12.40 (brs, 1H), 7.30 – 7.14 (m, 5H), 6.49 (s, 1H), 5.20 (t, J = 7.2 Hz, 1H), 3.84 (s, 3H), 3.32 – 3.26 (m, 4H), 2.94 - 2.89 (m, 2H), 1.75 (s, 3H), 1.63 (s, 3H) ppm.^{13}C NMR (75 MHz, acetone-d_6): δ = 174.4, 163.5, 162.3, 146.0, 143.1, 131.2, 129.2, 129.1, 126.6, 123.5, 115.3, 106.8, 105.3, 56.0, 39.9, 39.1, 25.9, 22.5, 17.8 ppm. HRMS (ESI): calcd for $C_{21}H_{24}O_4Na^+$ [M + Na]$^+$: 363.1567, found: 363.1568.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (Grant No. 81473081, 81673312), Natural Science Foundation of Jiangsu Province for outstanding young scholars (BK20160078), Scientific Research Foundation for the Returned Overseas Chinese Scholars, Ministry of Education (2013) and Technology Foundation for Selected Overseas Chinese Scholar, Ministry of Personnel of China (2013).

Keywords: Total synthesis • Natural product • Antitumor • *Pseudo*-Amorfrutin A • Amorfrutin C

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10.1002/ejoc.201701811

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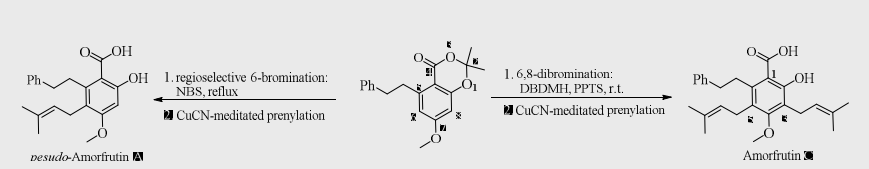

Total synthesis

Qi Miao, Yunzhi Li, Jinyi Xu, Aijun Lin, Genzoh Tanabe, Osamu Muraoka, Xiaoming Wu and Weijia Xie**

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First total syntheses of Amorfrutin C and *pseudo*-Amorfrutin A

Syntheses of Amorfrutin C as a potent antitumor natural product as well as *pseudo*-Amorfrutin A were accomplished. The 5 position bromination and 3,5-dibromination of **5** can be achieved via different bromination condition in high yield respectively. The introduction of prenyl moiety to**5** could be achieved through a subsequent CuCN-meditated alkylation reaction to give Amorfrutin C and *pseudo* Amorfrutin A in moderate yields.

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