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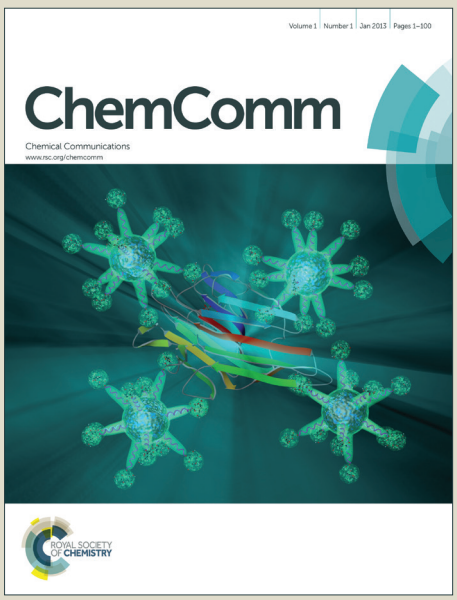

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ARTICLE TYPE

Total Synthesis of Astrosterioside A, an Anti-inflammatory Asterosaponin

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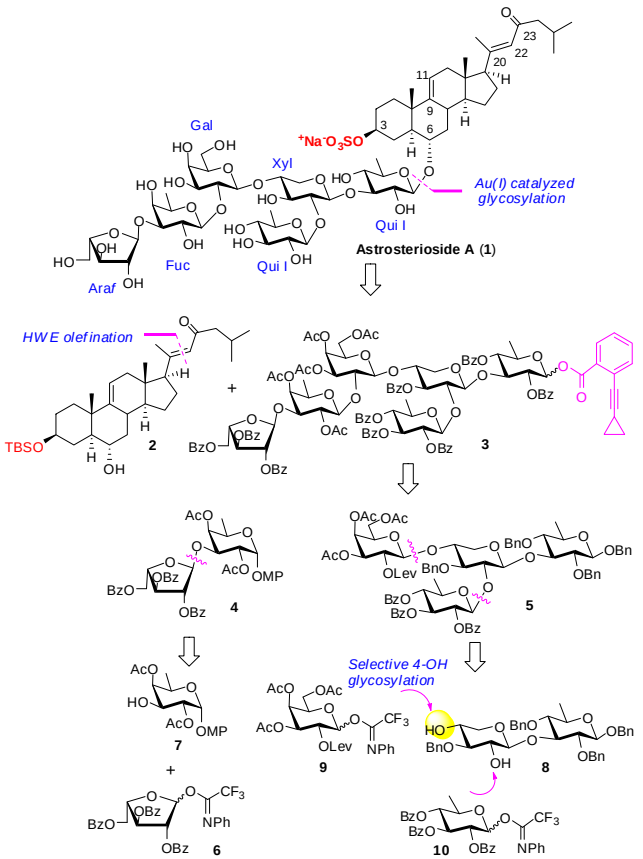
Astrosterioside A, a sulfated steroidal hexasaccharide isolated from starfish *Astropecten monacanthus* showing potent anti-inflammatory activity, was synthesized in a convergent linear sequence of 24 steps and in 6.8% overall yield from adrenosterone.

Asterosaponins are found ubiquitous in the marine Asteroidea species (starfish); thus far, over 100 such saponin compounds have been characterized from some 100 starfish species collected in all climatic areas.[1-3] Structurally, the asterosaponins share a $\Delta^{9(11)}$-3β,6α-dihydroxysteroidal nucleus with a sulfate residue at the C3 and a glycan at the C6. The glycans are mostly penta- or hexasaccharides containing all 1,2-*trans*-glycosidic linkages and a (1→2) branching residue at the second sugar unit. These secondary metabolites are belived to play an important role as defense chemicals to protect the slow-moving starfish from parasites and predators. Indeed, their cytotoxic, antimicrobial, and antifouling effects have been disclosed.[2,3] However, the in-depth studies on the activities of asterosaponins has been hampered by their poor accessibility. Chemical synthesis of these highly polar and complex glycoconjugates has been a formidable task;[4-9] the first total synthesis of an asterosaponin, namely goniopectenoside B, was achieved only recently.[10]

Astrosterioside A (**1**), featuring a 20(22)*E*-ene-23-one motif in the steroidal side chain and a terminal α-L-arabinofuranose in glycan, was characterized in 2013 from an edible Vietnamese starfish *Astropecten monacanthus* (Scheme 1).[11] This compound exhibited significant inhibitory effects on the production of pro-inflammatory cytokines IL-6 in LPS-stimulated bone marrow-derived dendritic cells (IC_{50} = 3.17 μM). The activity was found to be highly dependent on the steroidal side chain; its congeners Astrosteriosides C-D with varied side chains were much less active (IC_{50} > 30 μM). To facilitate further studies on the structure-activity relationship (SAR) and the mechanism of action, we embarked on the chemical synthesis of this type of asterosaponins. Here we report the first total synthesis of Astrosterioside A (**1**).



Scheme 1. Structure of astrosterioside A (**1**) and its retrosynthetic analysis.

To be convergent in the synthesis,[12] astrosterioside A (**1**) was disconnected into aglycon derivative **2** and hexasaccharide donor **3**, wherein the aglycon 3-OH was protected as a *tert*-butyldimethylsilyl (TBS) ether and the glycan hydroxyl groups were fully protected as acetyl or benzoyl esters to ensure the final introduction of the 3-*O*-sulfate residue and global deprotection (Scheme 1). The aglycon **2** could be derived from the commercially available adrenosterone via elaboration of the $\Delta^{9(11)}$-3β,6α-dihydroxysteroidal core and installation of the side chain using Horner–Wadsworth–Emmons (HWE) olefination.[13,14] The hexasaccharide donor **3** was functionalized with a *ortho*-(cyclopropylethynyl)benzoate at the anomeric carbon and equipped with a neighboring-participating benzoyl group at C2, thus the later-stage condensation of the glycan and the aglycon would be realized under the mild gold(I)-catalyzed conditions and

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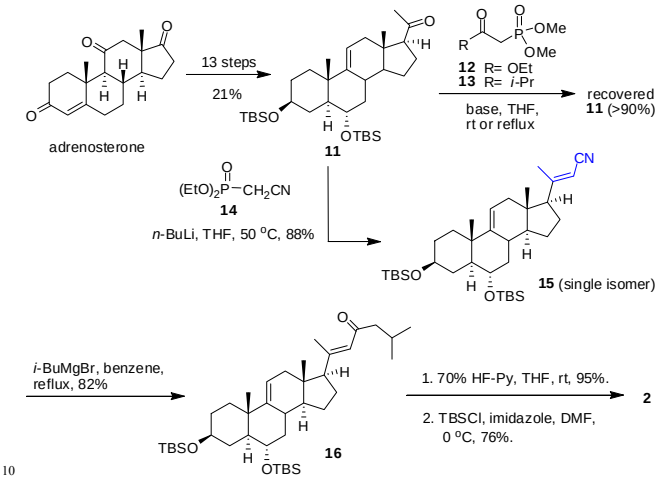
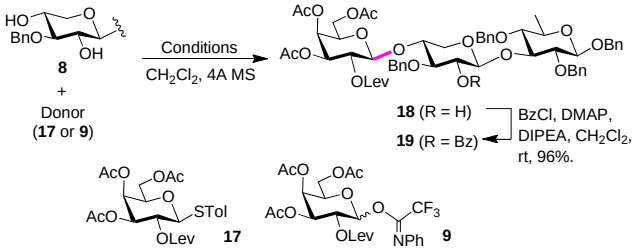
in a stereo-controlled manner.[15-18] The *ortho*-(cyclopropylethynyl)benzoate **3** was planned to be elaborated from a hexasaccharide precusor that could be assembled from five building blocks **6-10**, in that *N*-phenyl trifluoroacetimidate[19,20] was employed as the leaving group in the donors and neighboring-participating groups (i.e., acetyl, benzoyl, and levuloyl group) were installed to secure the formation of the 1,2-*trans*-glycosidic linkages.



Scheme 2. Synthesis of the aglycon derivative **2**.

Starting from adrenosterone, 3β,6α-di-*O*-TBS-$\Delta^{9(11)}$-20-keone **11** was prepared readily in 13 steps and 21% overall yield following literature procedures (Scheme 2).[10,21] Installation of the side chain onto ketone **11** by a HWE reaction with phosphonate **12** or **13**[22] was found unsuccessful, wherein ketone **11** was largely intact in the presence of a variety of bases, such as sodium hydride, lithium diisopropylamide (LDA), *n*-butyllithium, and potassium *tert*-butoxide. Gratifyingly, the olefination of **11** was achieved with diethyl cyanomethylphosphonate **14**[23,24] in the presence of *n*-butyllithium (THF, 50 °C), providing nitrile **15** in 88% yield as a single *E*-isomer. Subsequent addition of *iso*-butylmagnesium bromide to nitrile **15** in refluxing benzene gave enone **16** in 82% yield.[25] Removal of the two *O*-TBS groups with 70% HF·pyridine in THF, followed by the selective protection of the resultant 3β-OH with *tert*-butyldimethylsilyl chloride in dimethylformamide at 0 °C furnished the desired steroidal aglycon derivative **2** in a satisfactory 76% yield.[7] A strong NOESY correlation between H-22 (δ_H 6.07) and H-17 (δ_H 2.25) and no correlation between H-22 and H-21 (δ_H 2.14) were observed, confirming the *E* configuration of the C-20/C-22 double bond. Thus, the aglycon derivative **2** was synthesized in 17 scalable steps and 11% overall yield from the commercially available adrenosterone.

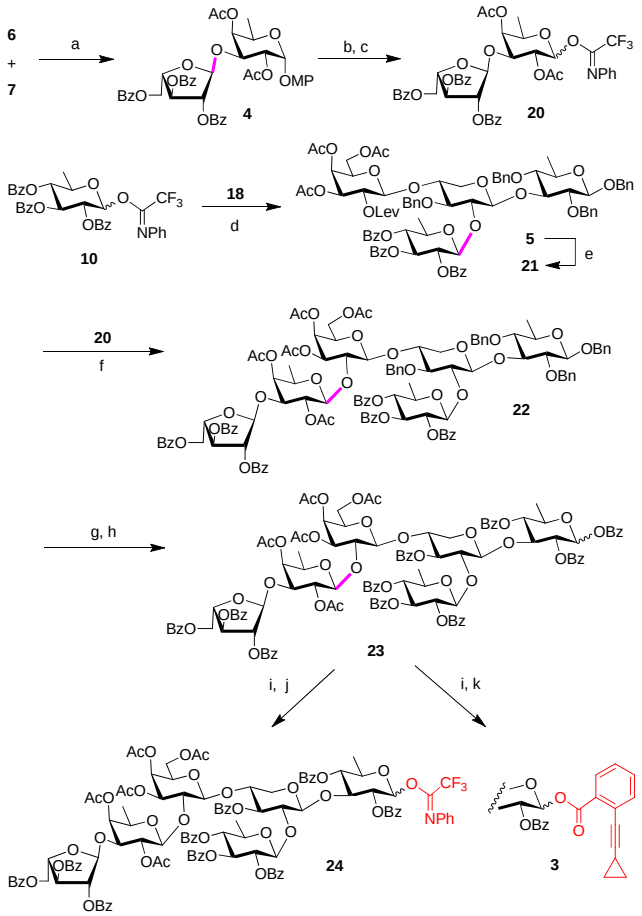


Entry	Donor	Promoter	T (°C)	Yield (**18**)
1	**17**	0.1 equiv. TMSOTf, 1.5 equiv. NIS	-30	68% *(2-O-glycosylation <5%)*
2	**17** (reverse addition)	0.05 equiv. TMSOTf, 1.5 equiv. NIS	-30	85%
3	**17**	1 equiv. BSP, 1.1 equiv. Tf_2O, 2 equiv. TTBP	-60	70%
4	**9**	0.05 equiv. TMSOTf	-30	92%

Scheme 3. Regio-selective 4-OH glycosylation for the preparation of trisaccharide **18**.

To enhance the efficiency in the assembly of the glycan, a regio-selective glycosylation of disaccharide diol **8** was explored (Scheme 3).[26,27] Thus, coupling of the xylopyranoside 2,4-diol **8** (1.5 equiv.) with thiogalacoside **17**[28] under the action of TMSOTf (0.1 equiv.) and *N*-iodosuccinimide (1.5 equiv.) at -30 °C afforded the desired β-(1→4)-coupled trisaccharide **18** in 68% yield (entry 1), with trace amount the β-(1→2)-coupled trisaccharide being detected. Reducing the amount of TMSOTf to 0.05 equivalent and reversing the addition sequence led to **18** in a better yield (85%; entry 2). The promotion system developed by Crich[29] (a combination of 1-benzenesulfinyl piperidine (BSP), trifluoromethanesulfonic anhydride (Tf_2O), and 2,4,6-tri-*tert*-butylpyrimidine (TTBP)) furnished trisaccharide **18** in 70% yield. With galactosyl *N*-phenyl trifluoroacetimidate **9** as donor, the coupling under the catalysis of TMSOTf (0.05 equiv.) provided **18** in an execellent 92% yield (entry 4).

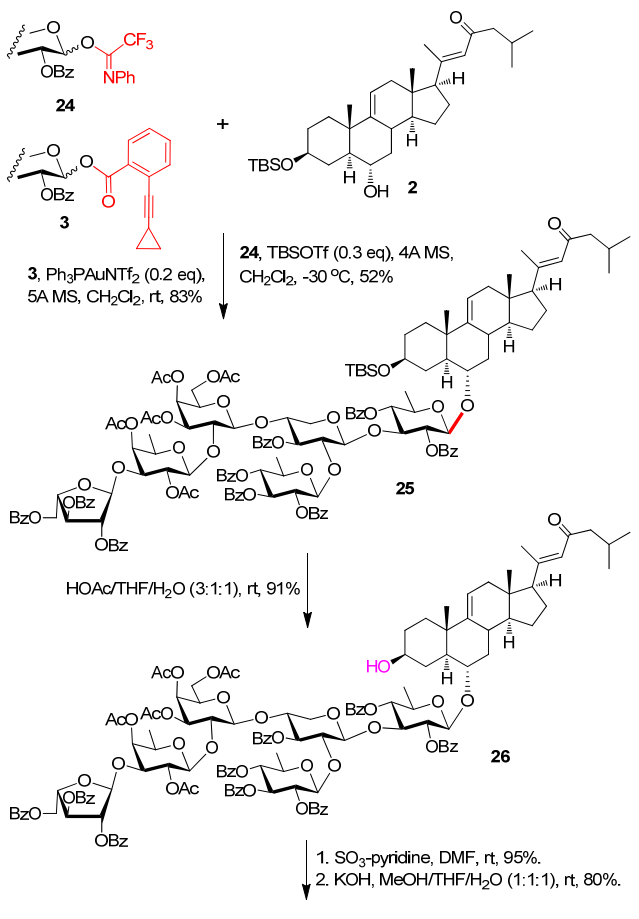
The structure of trisaccharide **18** was confirmed by careful interpretation of the COSY, HSQC, and HMBC NMR spectra. The chemical shifts of the three anomeric carbons in **18** were at 102.1 (C1), 103.8 (C1'), and 100.3 ppm (C1''), respectively, indicating the β configurations. The glycosylation position (at 4'-OH) in **18** was confirmed by ^{13}C NMR comparison with its benzoyl derivative **19**, of which the three anomeric carbon signals appeared at 102.0 (C1), 100.2 (C1'), and 100.8 ppm (C1''), respectively. The shift of C1' signal to a high field (by 3.6 ppm) was in agreement with the acylation at it neighboring 2'-OH.[30,31]



Scheme 4. Synthesis of the hexasaccharide *ortho*-(cyclopropylethynyl)benzoate **3** and *N*-phenyl trifluoroacetimidate **24**. (a) TMSOTf (0.1 equiv.), 4Å MS, CH_2Cl_2, -30 °C, 95%. (b) Ceric ammonium nitrate, CH_3CN/H_2O (4:1), rt, 83%. (c) 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride, K_2CO_3, acetone, rt, 98%. (d) **18**, TMSOTf (0.2 equiv.), 4Å MS, CH_2Cl_2, 0 °C-rt, 91%. (e) $NH_2NH_2 \cdot H_2O$, pyridine/AcOH (3:2), rt, 95%. (f) **20** (1.3 equiv.), TMSOTf (0.1 equiv.), 4Å MS, CH_2Cl_2, -30 °C, 92%. (g) 20% $Pd(OH)_2/C$, H_2 (1 atm), EtOAc/EtOH (1:1), rt, 98%. (h) BzCl, DMAP, pyridine, 0 °C -rt, 96%. (i) $NH_2(CH_2)_2NH_2$, HOAc, THF, rt, 72% (with 18% **23** recovered). (j) *ortho*-(cyclopropylethynyl)benzoic acid, EDCI, DMAP, DIPEA, CH_2Cl_2, rt, 96%. (k) 2,2,2-trifluoro-*N*-phenyl acetimidoyl chloride, K_2CO_3, acetone, rt, 97%.

The remaining less reactive 2'-OH in **18** was then glycosylated with perbenzoyl D-quinovopyranosyl imidate **10**[28] under stronger reaction conditions (0.2 equiv. TMSOTf, 4Å MS, CH_2Cl_2, 0 °C-rt), leading to the desired tetrasaccharide **5** in a high 91% yield (Scheme 4). The Lev group in **5** was selectively removed with hydrazine hydrate to provide **21** (95%). Meanwhile, the terminal β-L-Ara*f*-(1→3)-D-Fuc*p* building block **4** was synthesized by coupling of fucose acceptor **7** with arabinofuranosyl *N*-phenyl trifluoroacetimidate **6** under the catalysis of TMSOTf (CH_2Cl_2, 4Å MS, -30 °C; 95%). Disaccharide **4** was then converted into *N*-phenyl trifluoroacetimidate **20** in a two-step sequence, involving selective removal of the anomeric 4-methoxyphenol group (CAN, CH_3CN/H_2O, 83%) and condensation with 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (K_2CO_3, acetone, 98%).[19,20] Condensation of tetrasaccharide **21** with disaccharide imidate **20** was realized under the catalytic of TMSOTf (0.1 equiv.) in CH_2Cl_2 at -30 °C, furnished the desired hexasaccharide **22** in a satisfactory 92% yield.

At this stage, the protecting groups in hexasaccharide **22** were synchronized with acyl groups. Thus, the four benzyl groups were hydrogenolysed over $Pd(OH)_2/C$ and the resultant hydroxyl groups were protected with benzoyl groups, providing hexasaccharide **23** (94% over two steps). Hexasaccharide **23** was then transformed into the corresponding *ortho*-(cyclopropylethynyl)benzoate **3** and *N*-phenyl trifluoroacetimidate **24** in two-step sequences, i.e., selective removal of the anomeric benzoyl group ($NH_2(CH_2)_2NH_2$, HOAc, THF, 72% yield)[32] followed by condensation with *ortho*-(cyclopropylethynyl)benzoic acid (EDCI, DMAP, DIPEA, CH_2Cl_2, 96%)[33] or with 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (K_2CO_3, acetone, 97%).



Scheme 5. Completion of the total synthesis of astrosterioside A (**1**).

With the hexasaccharide donors (**3** and **24**) and aglycon acceptor (**2**) in hand, the formation of the key glycosidic linkage was investigated (Scheme 5). The coupling of steroidal C6-OH **2** (2.0 equiv.) with hexasaccharide *N*-phenyl trifluoroacetimidate **24** under the catalysis of TBSOTf (0.3 equiv.) in the presence of 4Å molecular sieves in CH_2Cl_2 at -30 °C provided the desired β-

glycoside **25** in 52% yield, wherein the C6-*O*-TBS derivative and the hydrolyzed donor were isolated as the major byproducts. The coupling of aglycon **2** (3.0 equiv.) with hexasaccharide *ortho*-(cyclopropylethynyl)benzoate **3** proceeded smoothly under the catalysis of PPh_3AuNTf_2 (0.2 equiv.) in the presence of 5Å molecular sieves in CH_2Cl_2 at room temperature, affording the desired glycoside **25** in a satisfactory 83% yield; in addition, the excess aglycon **2** could be fully recovered. Removal of the 3-*O*-TBS group in **25** was effected with HOAc/THF/H_2O (3:1:1) at room temperature to provide **26** in 91% yield. Sulfation of the resulting C3-OH in **26** was achieved over sulfur trioxide-pyridine in dimethylformamide, leading to the 3-*O*-sulfate derivative,[7,10] which was subjected to global removal of the acetyl and benzoyl groups (KOH, MeOH/THF/H_2O, rt) to provide astrosterioside A (**1**) in a good 80% yield. The analytical data of **1** are in good agreement with those reported for the natural product.[11,34]

Summarizing, we have accomplished the first total synthesis of astrosterioside A (**1**), a characteristic asterosaponins with potent inflammatory activities, with a convergent linear sequence of 24 steps and in a high 6.8% overall yield. The synthesis features a stereoselective HWE olefination to construct the steroidal 20(22)*E*-ene-23-one side chain (**11**+**14**→**15**), a regioselective glycosylation of the xylopyranoside 4-OH (**8**+**9**→**18**), and a highly efficient gold(I)-catalyzed coupling of the aglycon with hexasaccharide (cyclopropylethynyl)benzoate donor (**3**+**2**→**25**). Given the conserved nature of the structures of asterosaponins, the work reported here shall facilitate the synthesis of other asterosaponins and thus the in-depth studies on their biological and pharmacological activities.

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