Structure and Total Synthesis of HF-7, a Neuroactive Glyconucleoside Disulfate from the Funnel-Web Spider *Hololena curta*

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Abstract: Spider venom toxins have attracted considerable attention for their ability to block the action of excitatory amino acids such as glutamate and aspartate. A new neuroactive compound designated HF-7 was isolated in 1993 from the venom of a funnel-web spider, *Hololena curta*. HF-7 was shown to block kainate receptors and, albeit weakly, L-type calcium channels. Spectroscopic analysis established the structure of HF-7 as an unusual acetylated, disulfated fucopyranosyl guanosine, with the acetate ester attached at the 4-position of an α-linked fucose ring and two sulfates attached to the ribose ring. Because insufficient quantities of natural HF-7 were available for chemical degradation or X-ray diffraction analysis, total synthesis of three candidate structures was used to establish the identity of HF-7. Once HF-7 was fully characterized as 3′-*O*-(4″-*O*-acetyl-α-L-fucopyranosyl)guanosine-2′,5′-disulfate, an improved, targeted synthesis of the natural product was developed.

The remarkable dominance of insects and other arthropods on land can be attributed, in part, to the extraordinary diversity of their chemical defense mechanisms.[1] In addition to glandular defensive secretions and systemic antifeedants, some anthropods muster offensive chemical weaponry to capture their prey. In this regard, spider venom toxins have attracted considerable attention for their ability to block the action of excitatory amino acids such as glutamate and aspartate.[2] Receptors for such amino acids (NMDA, kainate, quisqualate/AMPA) and their closely associated ion channels affect the intracellular concentration of Ca^{2+} in nerve cells and are important in a variety of neural functions, including pain,[3] motor control,[4] learning, and memory.[5]

Most spider-derived toxins are characterized by polypeptide, protein, or polyamine backbones.[6] As part of a collaboration between scientists at Cornell University and Cambridge NeuroScience, Inc., a new neuroactive compound designated HF-7 was isolated in 1993 from the venom of a funnel-web spider, *Hololena curta*.[7] This highly polar agent was shown to block kainate receptors and, albeit weakly, L-type calcium channels. Such agents might be used in treating global cerebral ischemia, a form of excitotoxicity mediated by non-NMDA receptors that can arise following cardiac arrest, drowning, or carbon monoxide poisoning.[8]

From initial spectroscopic analysis, it was clear that HF-7 belonged to no known class of spider venoms. The presence of a guanine chromophore was established by ultraviolet absorption spectroscopy. Satisfactory mass spectrometric data could only be obtained by negative ion fast atom bombardment (FAB) mass spectrometry, which revealed a parent monoanion at *m*/*z* 630 corresponding to a molecular weight of 631 Da. The presence of a sulfate group was confirmed by loss of an 80 Da fragment from this anion, corresponding to the loss of SO_3 from the parent ion. Exact mass measurement of the resulting base peak in the spectrum established the molecular formula $C_{18}H_{24}N_5O_{13}S$ (*m*/*z* obsd 550.1036; calcd 550.1091), from which the molecular formula for HF-7 was inferred to be $C_{18}H_{24}N_5O_{16}S_2$. Further spectroscopic analysis established the structure of HF-7 as an unusual acetylated, disulfated fucopyranosyl guanosine, with the acetate ester attached at the 4-position of an α-linked fucose ring and two sulfates attached to the ribose ring.

Several aspects of the overall structure of HF-7 are noteworthy. To our knowledge, HF-7 is, together with liposidomycin,[9] the only known naturally occurring sulfated nucleosides. Related anionic nucleosides include 3′-phospho-adenosine-5′-phosphosulfate and the sulfamoylated nucleosides ascamycin,[10] AT-265,[11] and nucleocidin.[12] HF-7 also belongs to a very small

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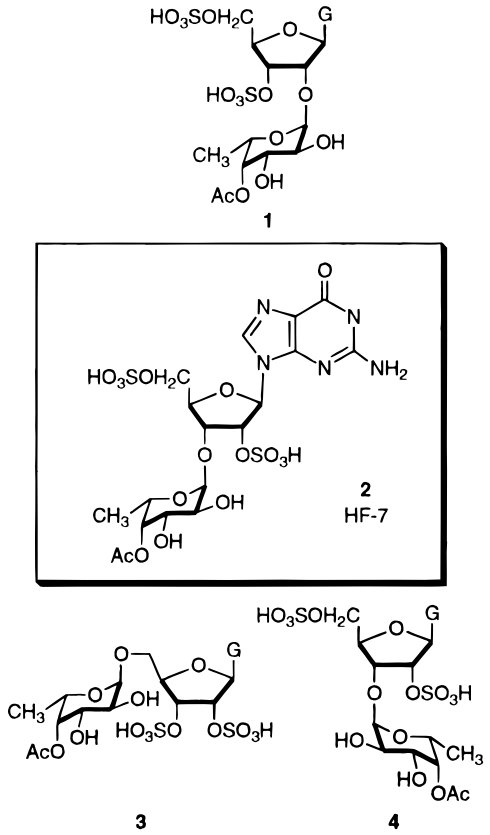
Scheme 1

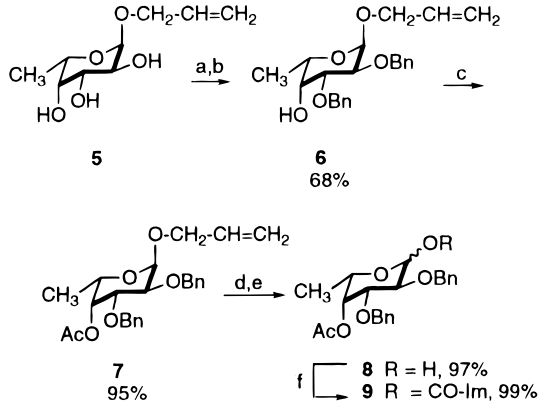


family of glycosylated nucleosides[13] and contains the more unusual α-glycosidic linkage. Other known examples are the fucosylated nucleoside shimofuridins A−G[14] and the phosphorylated nucleoside glycosides adenophostin A and B, which also affect Ca^{2+} release.[15]

Limited amounts of natural HF-7 available precluded the use of ^{1}H-detected (^{1}H, ^{13}C) multiple bond correlation (HMBC) NMR spectroscopy to identify the site of the fucose linkage, and hence the sites of sulfation on the ribose ring. With insufficient quantities for chemical degradation or X-ray diffraction analysis, the absolute configuration of the fucose and ribose could not be determined. Since a D- or L-fucose could be joined at the 2′-, 3′-, or 5′-positions of a D- or L-ribose, 12 candidates were possible for the structure of HF-7. Assuming utilization of the more common enantiomers of monosaccharides (D-ribose and L-fucose), the number of possibilities was reduced to three (**1**−**3**, Scheme 1).

In such a situation, total synthesis of the candidate structures represents a useful way to complete the characterization of a new natural product, as well as to obtain adequate quantities of both the natural material and some closely related analogues for further biological evaluation. To establish the structure of HF-7, we herein report the synthesis of four acetylated, disulfated fucosylguanosines: 2′-*O*-(4″-*O*-acetyl-α-L-fucopyranosyl)guanosine-3′,5′-disulfate (**1**), 3′-*O*-(4″-*O*-acetyl-α-L-fucopyranosyl)guanosine-2′,5′-disulfate (**2**), 5′-*O*-(4″-*O*-acetyl-α-L-fucopyranosyl)guanosine-2′,3′-disulfate (**3**), and 3′-*O*-(4″-*O*-acetyl-α-D-fucopyranosyl)guanosine-2′,5′-disulfate (**4**). On the basis of spectroscopic comparisons with the natural product, the structure of HF-7 was shown to be (−)-**2** (Scheme 1). Following preliminary disclosure of the isolation and characterization of HF-7,[1] an efficient route specifically designed for the regio- and stereoselective synthesis of (−)-**2** itself was developed.

Scheme 2[a]



[a] Conditions: (a) Bu_2SnO, toluene, reflux, 16 h; (b) BnBr, DMF, 90 °C, 9 h; (c) Ac_2O, pyr, room temperature, 16 h; (d) $(Ph_3P)_3RhCl$, EtOH, reflux, 22 h; (e) HgO, $HgCl_2$, 9:1 acetone/H_2O, room temperature, 2 h; (f) carbonyldiimidazole, ether, room temperature, 1 h.

One synthetic approach to regioisomers **1**−**3** relied on coupling guanosine derivatives suitably blocked at the 2′-, 3′-, or 5′-positions with an acetylated and appropriately protected fucopyranosyl donor. Several multistep procedures for distinguishing the hydroxyl groups of L-fucose have been developed in synthetic studies on blood-group determinants.[16] Guided ultimately by the requisite compatibility (and selective cleavage) of protecting groups in the two saccharide subunits, we implemented an expeditious route to 4-*O*-acetyl-2,3-di-*O*-benzyl-1-*O*-imidazolylcarbonyl-α-L-fucopyranoside (**8**), depicted in Scheme 2. Reactions of the known allyl α-L-fucopyranoside (**5**)[17] with 2 equiv of Bu_2SnO in toluene, followed by heating with excess BnBr in DMF, afforded 2,3-dibenzyl ether **6** in 68% yield. A similar, tin-mediated 2,3-di-*O*-benzylation of methyl α-D-galactopyranoside (13% yield) was recently reported.[18] The mechanism of this reaction, although unknown, was suggested to involve initial benzylation at O2, followed by benzylation at O3. Acetylation of **6** afforded **7**, which, upon subsequent deallylation using Wilkinson's catalyst followed by $HgCl_2$/HgO, afforded **8**. Compound **8** was transformed into imidazolylcarbonyl glycoside **9**. We chose this type of glycosylating agent on the basis of its successful use in the synthesis of avermectin by Ley and Ford,[19] who found that it worked well in the construction of a hindered glycoside, and that it strongly favored α-glycosylation.

Scheme 3 depicts the synthesis of appropriate guanosine building blocks **12** and **13** used to prepare target structures **1**−**4**.

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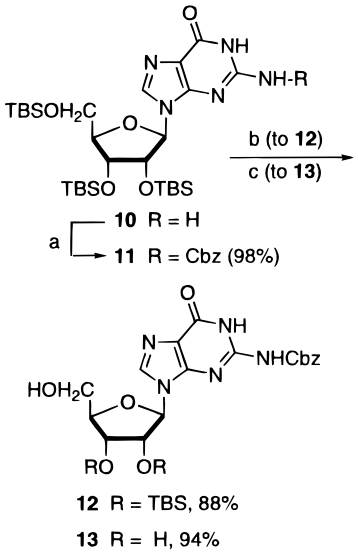
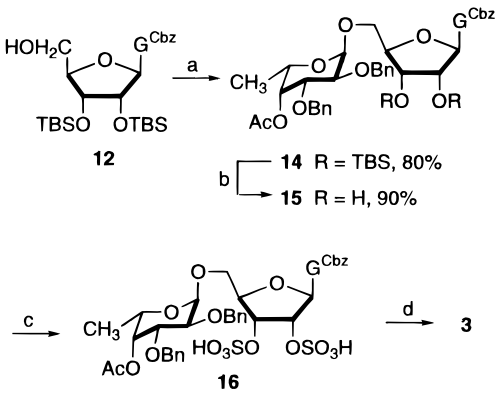
Scheme 3[a]



[a] Conditions: (a) Cbz-imidazole, KH, 18-crown-6, THF, 2 h; (b) 1:1 TFA/CH_2Cl_2, 2 h; (c) Bu_4NF, THF, 20 h.

Scheme 4[a]

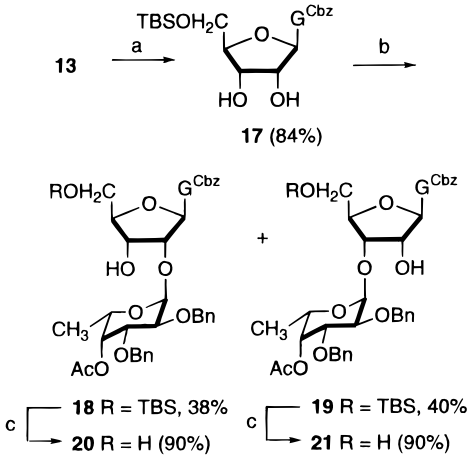


[a] Conditions: (a) **9**, CH_2Cl_2, $ZnBr_2$; (b) Bu_4NF, THF; (c) SO_3·DMF; (d) H_2, $Pd(OH)_2$, 23% from **15**.

The *N*-carbobenzoxy group (Cbz) was chosen to protect the guanine's primary amine group during the glycosylation step, since both the Cbz and fucose benzyl ethers could subsequently be removed by hydrogenolysis under conditions that would not deacetylate the fucose ring. Only one report appeared on the use of Cbz group to protect the exocyclic amino group of nucleoside bases. The paper described a lengthy route to the *N*-Cbz derivative of 2-deoxyguanosine, but not of guanosine itself.[20] Therefore, the approach indicated in Scheme 3 was developed. Reaction of the known[21] tris-silyl ether **10** with Cbz-imidazole[22] afforded the Cbz-protected nucleoside **11** in excellent yield. Selective deprotection of **11** to alcohol **12** was achieved using 1:1 TFA/CH_2Cl_2, whereas exhaustive desilylation with fluoride afforded N^{Cbz}-guanosine (**13**).

With appropriate fucose and guanosine building blocks in hand, a regioselective synthesis of 5′-*O*-(4″-*O*-acetyl-α-L-fucopyranosyl)guanosine-2′,3′-disulfate (**3**) was developed, as shown in Scheme 4. Fucosylation of guanosine selectively at the 5′-position was achieved by $ZnBr_2$-catalyzed coupling of bis-silyl ether **12** with the activated fucosyl donor **9** to afford α-linked glycoside **14** as the exclusive product. Fluoride-induced desilylation of **14** furnished diol **15**, which was smoothly sulfated using excess SO_3·DMF complex to give disulfate **16**. Purification at this stage either by HPLC or by ion-exchange chromatography was difficult because of the amphiphilic characteristics of **16**. Deprotection of **16** by exhaustive hydrogenolysis on $Pd(OH)_2$, followed by HPLC purification (reversed-phase, H_2O/CH_3CN/TFA), furnished the desired compound **3**. From the ^1H NMR spectrum of **3**, it was evident that H2′ and H3′ on the ribose ring were deshielded by 1.14 and 1.12 ppm, respectively, compared to the corresponding resonances in **15**, as would be expected from sulfation at the 2′- and 3′-hydroxyl groups. Moreover, the negative ion FAB mass spectrum revealed fragments at *m*/*z* 652 (M − 2H + Na) and 630 (M − H) in support of its assigned structure. However, comparison of the NMR spectrum of synthetic **3** with that of HF-7 revealed that the two compounds were not identical.

Scheme 5[a]



[a] Conditions: (a) TBSCl, imidazole, DMF, 2 h; (b) **9**, $ZnBr_2$, CH_2Cl_2, reflux, 1 h; (c) Bu_4NF, THF, 20 h.

Turning next to glycosylated nucleosides **1** and **2** as candidates for the structure of HF-7, the direct coupling of **8** with guanosine 2′,3′-diol **17** (Scheme 5) was investigated as an expedient approach to both **1** and **2**. Ether **17**, prepared by monosilylation of **13**, underwent a smooth, $ZnBr_2$-catalyzed reaction with **8** in CH_2Cl_2 at reflux to afford both the 2′- and 3′-coupled products **18** and **19** in 38% and 40% yields, respectively. In addition, the corresponding 2′,3′-difucosylated nucleoside (structure not shown) was obtained in 8−10% yield. Both **18** and **19** were readily obtained pure by flash column chromatography, and each could be desilylated in excellent yield to afford **20** and **21**, respectively. Structural assignments were confirmed at this stage by HMBC NMR spectroscopy, which indicated long-range coupling of the fucose anomeric hydrogen H1″ with the C2′ carbon of **20** and with the C3′ carbon of **21**. The corresponding couplings of the fucose C1″ with H2′ of **20** and H3′ of **21** were also observed.

The 2′-fucosylated nucleoside **20** underwent sulfation using excess SO_3·DMF to afford disulfate **22**, which, without purification, was hydrogenolyzed over $Pd(OH)_2$ to afford 2′-*O*-(4-*O*-acetyl-α-L-fucopyranosyl)-guanosine-3′,5′-disulfate (**1**) in 36% yield (Scheme 6). Examination of the NMR spectrum of **1**, taken after HPLC purification, revealed significant differences from that of authentic HF-7 under the same conditions.

By analogy with **20**, the major coupling product, 3′-fucosylated nucleoside **21**, was sulfated, and the resulting

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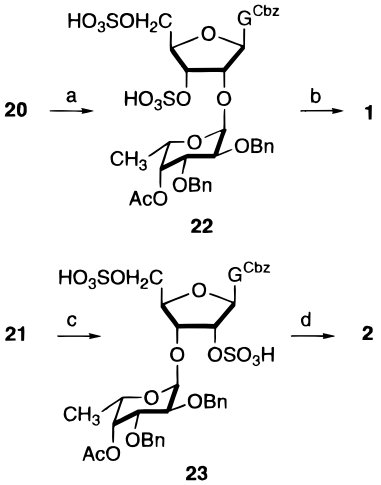
Scheme 6[a]



[a] Conditions: (a) SO_3·DMF, 0 °C to room temperature, 21 h; (b) H_2, $Pd(OH)_2$, 1:1 EtOH/H_2O, room temperature, 84 h, 36% from 20; (c) SO_3·DMF, room temperature, 2 h, 99%; (d) H_2, $Pd(OH)_2$, 12:12:1 CH_3OH/H_2O/TFA, room temperature, 16 h, 95%.

disulfate **23** was deprotected by hydrogenolysis to afford 3′-*O*-(4″-*O*-acetyl-α-L-fucopyranosyl)guanosine-2′,5′-disulfate (**2**) (Scheme 6). The high-resolution MS and UV spectra, as well as ^1H and ^{13}C NMR spectra (D_2O−TFA, pH 1.8), of synthetic **2** matched those of natural HF-7. Moreover, the ^1H NMR spectrum of a 1:1 mixture of synthetic **2** and HF-7 was indistinguishable from spectra of each pure component. Taken as a whole, the spectroscopic data indicated that the structure of HF-7 was either **2** or its mirror image.

Synthetic **2** derived from L-fucose and D-guanosine was levorotatory. However, the limited quantity of HF-7 available made it impossible to obtain a reliable specific rotation of the natural product for direct comparison with that of (−)-**2**. Unfortunately, neither the natural product nor synthetic (−)-**2** displayed any Cotton effect (200−350 nm) in its circular dichroism spectrum.

An exhaustive literature search uncovered no naturally occurring nucleoside or nucleotide that incorporated L-ribose. To ascertain whether the hexose in naturally occurring HF-7 possessed the D-configuration, the synthesis of 3′-*O*-(4″-*O*-acetyl-α-D-fucopyranosyl)guanosine-2′,5′-disulfate (**4**) was carried out using the enantiomer of **9** (Scheme 2) prepared from D-fucose. As expected, the ^1H NMR spectra of synthetic **4** and natural HF-7 showed distinct, though minor, differences, leading to the conclusion that HF-7 has the structure and absolute configuration depicted in **2**.

With HF-7 fully characterized, it became possible to consider an improved, targeted synthesis of (−)-**2**. First we explored the possibility of using 2-*N*-benzyloxycarbonyl-2′,5′-di-*O*-*tert*-butyldimethylsilyl-guanosine (**27**) as acceptor molecule in the fucosylation reaction. Direct disilylation of N^{Cbz}-guanosine (**13**) with a slight excess of TBSCl afforded mixtures of the 2′,5′- and 3′,5′-bis-silyl ethers in approximately a 1:1 ratio. Alternatively, guanosine was found to react with BOC anhydride selectively to afford the 3′-protected nucleoside **24** (Scheme 7). Silylation of the remaining free hydroxyl group using TBSCl gave nucleoside **25**, which upon reaction with Cbz-imidazole was transformed into the fully protected guanosine derivative **26**. The BOC group was removed selectively using TMSOTf-collidine[23] to afford **27**, the desired precursor to 3′-glycosylated

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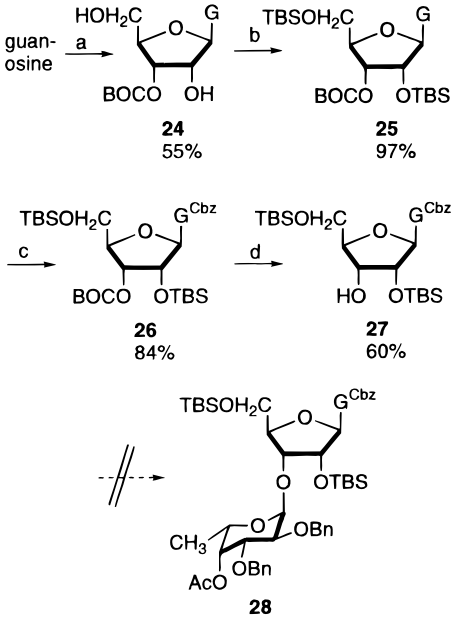
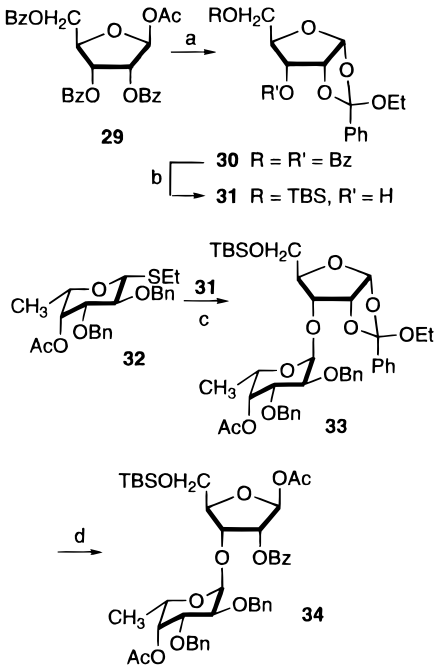
Scheme 7[a]



[a] Conditions: (a) BOC-anhydride, DMAP, Et_3N, DMSO, room temperature, 4 h; (b) TBSCl, imidazole, DMF, room temperature, 12 h; (c) Cbz-imidazole, KH, 18-crown-6, THF, room temperature, 2 h; (d) TMSOTf-collidine, CH_2Cl_2, room temperature, 1 h.

Scheme 8[a]

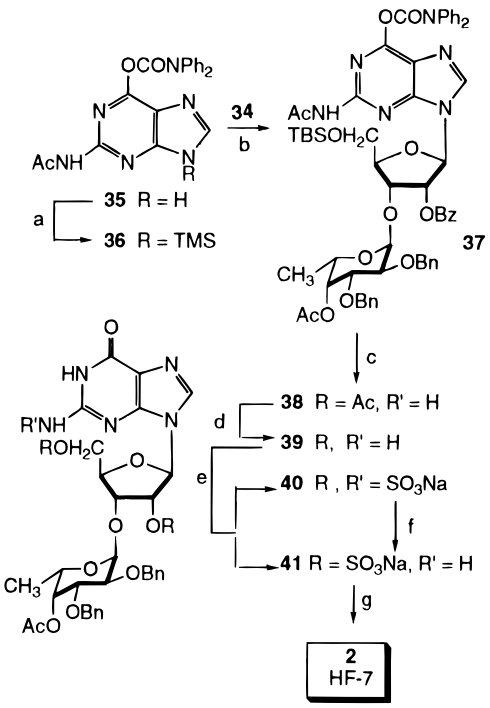


[a] Conditions: (a) (i) HCl, CH_2Cl_2, 0 °C, 2 h; (ii) $(CH_3)_3NCH(OEt)_2$, CH_2Cl_2, 18 h, 93%; (b) (i) $NaOCH_3$, CH_3OH, 2 h; (ii) TBSCl, CH_2Cl_2, pyridine, Et_3N, 4 h, 84%; (c) IDCP, 4:1 DCE/Et_2O, 3 h; (d) HOAc, 15 h, 75% from **31**.

nucleoside **2**. However, attempts to bring about the reaction of **27** with fucosyl donor **9** using a variety of catalysts afforded none of the desired coupling product **28**.

The inability to fucosylate guanosyl acceptor **27** was an incentive to develop an entirely different strategy to synthesize **2** via a sequential stepwise introduction of the *O*- and *N*-glycosidic linkages, followed, after protecting group manipulations, by sulfation and deprotection. Formation of the *O*-glycosidic linkage commenced as depicted in Scheme 8, with the

Scheme 9[a]



[a] Conditions: (a) **35**, BSA, DCE, 80 °C, 15 min; (b) **34**, TMSOTf, toluene, 80 °C, 3 h (two steps, 80%); (c) (i) $3HF{\cdot}Et_3N$, pyridine, 6 h; (ii) 25% NH_4OH, CH_3OH, 60 °C, 24 h; (iii) Ac_2O, pyridine, 8 h (three steps, 67%); (d) 0.02 M K_2CO_3, 2:1 CH_3OH/CH_2Cl_2, 22 h; (e) $SO_3{\cdot}$pyr, pyridine, 55 °C, 4 h; (f) 0.2 N H_2SO_4, 12 h (95% based on **37**); (g) H_2, 10% $Pd(OH)_2/C$, 10:5:1 $CH_3OH/H_2O/AcOH$, 15 h, 99%.

transformation[24] of commercially available ribofuranose **29** into the rigid 1,2-ortho ester **30**. Debenzoylation of **30** and subsequent regioselective silylation with TBSCl gave the partially protected ribosyl acceptor **31**. Fucosylation of the exposed 3-OH group in **31** with a slight excess of the highly potent and strongly α-directing ethyl 4-*O*-acetyl-2,3-di-*O*-benzyl-1-deoxy-1-thio-β-L-fucopyranoside (**32**)[25] under the influence of the mild promoter iodonium di-*sym*-collidine perchlorate (IDCP) yielded, after flash chromatography, the α-linked coupling product **33**, contaminated with a small amount of **32**. Acid-mediated ring-opening of the 1,2-ortho ester **33** under anhydrous conditions afforded β-acetate derivative **34**, thus setting the stage for the introduction (Scheme 9) of the β-linked guanyl moiety according to the well-known[26] Vorbrüggen procedure. Thus, TMSOTf-assisted condensation of **34** with persilylated guanine derivative **36**, prepared in situ by treatment of known[27] 2-*N*-acetyl-6-*O*-(diphenylcarbamoyl)guanine (**35**) with bis(trimethylsilyl)acetamide, proceeded as expected to afford glycoside **37**. Prior to the installation of the requisite 2′,5′-di-*O*-sulfate groups, **37** was subjected to the following sequence of protecting group manipulations. Desilylation of **37** with fluoride followed by complete deacylation and subsequent selective *O*-acetylation afforded crystalline **38**, having a free exocyclic amino function. Selective deacetylation[25] of **38** under mild basic conditions led to the isolation of diol **39**, which was subjected to sulfation using pyridine−sulfur trioxide complex. Workup and analysis of the reaction mixture by ^1H NMR spectroscopy revealed the presence of the required di-*O*-sulfated product **41** and a major amount (90%) of the corresponding trisulfate **40**. Unfortunately, attempts to suppress the unwanted sulfation of the exocyclic amino function in **39** were abortive. However, selective *N*-desulfation[28] could be accomplished under acidic conditions (i.e., 0.2 N H_2SO_4, 18 h) without affecting the integrity of the glycosidic linkages, as gauged by ^1H NMR spectroscopy. Accordingly, exposure of a mixture containing **40** and **41** (9:1 ratio) to the same acidic conditions gave, after ion-exchange (Dowex Na^+ form) and purification (Sephadex LH-20), the homogeneous di-*O*-sulfated product **41** in an excellent yield. Hydrogenolysis of **41** over $Pd(OH)_2$ on carbon led, without further purification, to the isolation of HF-7 (Na^+ salt) in near-quantitative yield. Synthetic HF-7 was levorotatory and indistinguishable from the natural material by reversed-phase HPLC, NMR spectroscopy, and mass spectrometry.

In conclusion, the results described in this paper characterize HF-7, a new neuroactive compound, as 3′-*O*-(4″-*O*-acetyl-α-L-fucopyranosyl)guanosine-2′,5′-disulfate (**2**) and detail two independent syntheses of this novel spider venom component.

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Supporting Information Available: Experimental procedures as well as spectroscopic and physical characterization data for all new compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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