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Synthesis of Selenium Analogues of the Naturally Occurring Glycosidase Inhibitor Salacinol and Their Evaluation as Glycosidase Inhibitors†

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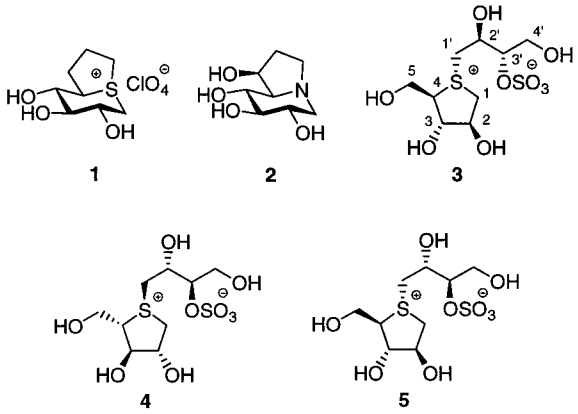
Abstract: The syntheses of two selenium analogues (**10** and **11**) of the naturally occurring sulfonium ion, salacinol (**3**), are described. Salacinol is one of the active principles in the aqueous extracts of *Salacia reticulata* that are traditionally used in Sri Lanka and India for the treatment of diabetes. The synthetic strategy relies on the nucleophilic attack of a 2,3,5-tri-*O*-benzyl-1,4-anhydro-4-seleno-D-arabinitol at the least hindered carbon of benzyl- or benzylidene-protected D- or L-erythritol-1,3-cyclic sulfate. The use of 1,1,1,3,3,3-hexafluoro-2-propanol as a solvent in the coupling reaction proves to be beneficial. Enzyme inhibition assays indicate that **10** is a better inhibitor ($K_i = 0.72$ mM) of glucoamylase than **3**, which has a K_i value of 1.7 mM. In contrast, **11** showed no significant inhibition of glucoamylase. Compounds **10** and **11** showed no significant inhibition of barley-α-amylase or porcine pancreatic-α-amylase.

Introduction

Glycomimetics in which specific oxygen atoms of carbohydrates have been replaced by different heteroatoms have been intensely investigated in the search for novel glycosidase inhibitors.[1] Our efforts in recent years have focused on a novel class of glycosidase inhibitors containing sulfonium ions as putative mimics of the oxacarbenium ion intermediates in glycosidase hydrolysis reactions.[2,3] Thus, we have described the synthesis and conformational analysis of a sulfonium ion analogue (**1**) of the naturally occurring glycosidase inhibitor of the indolizidine alkaloid class, castanospermine (**2**) (Chart 1).[2]

Yoshikawa et al.[4] also described the isolation of a naturally occurring glycosidase inhibitor containing a zwitterionic sulfonium−sulfate structure, namely salacinol (**3**), from the plant *Salacia reticulata*, which prompted us[3] and others[5] to synthesize **3** and its stereoisomers, **4** and **5** (Chart 1), and provide

Chart 1



conclusive proof of structure of the natural product. We have also reported the syntheses of the corresponding nitrogen congeners **6** and **7** (Chart 2) as potential glycosidase inhibitors,[6] and this has been followed by a similar report from another group.[7] We have also reported the syntheses of 1,4-anhydro-D-xylitol heteroanalogues **8** and **9** (Chart 2) and their evaluation as glycosidase inhibitors.[8] Most recently, Yoshikawa et al.[9] reported the inhibitory activity of **3** against several α-glucosidases and also the inhibitory effects of extracts of *Salacia*

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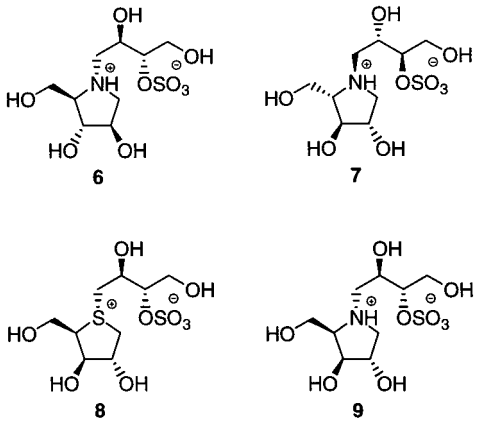
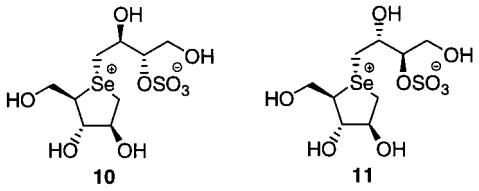
Chart 2



reticulata on serum blood glucose levels in maltose- and sucrose-loaded rats. Those authors also assigned the "absolute stereostructure" of **3** (Chart 1) to a zwitterionic compound comprised of a 1,4-anhydro-4-thio-D-arabinitol moiety and a 1′-deoxy-D-erythrosyl-3′-sulfate unit.[9] However, we point out here that carbohydrate nomenclature dictates that the latter unit be designated a 1-deoxy-L-erythrosyl-3′-sulfate unit, as also proven by our earlier work on the synthesis of the different stereoisomers of salacinol.[3]

As part of our continuing interest in evaluating the effect of the heteroatom substitution in the sugar ring on glycosidase inhibitory activity, we report in the present work the synthesis of novel analogues of **3** and its diastereomer **5**, in which the sulfur atom has been replaced by the heavier cognate atom, selenium, to give **10** and **11**, respectively (Chart 3). We report also their evaluation as glycosidase inhibitors of porcine pancreatic α-amylase (PPA), barley α-amylase (AMY1), and glucoamylase G2.

Chart 3

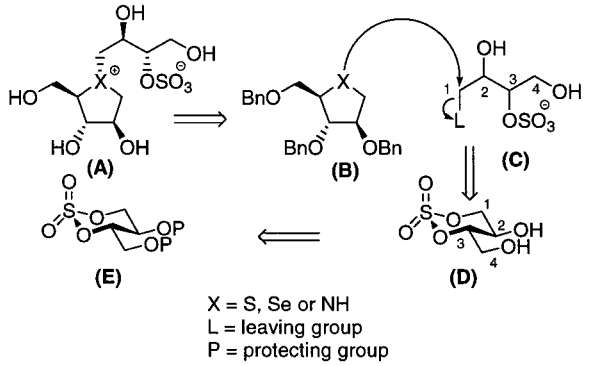


Results and Discussion

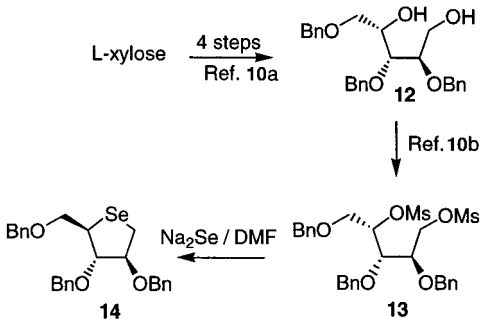
Retrosynthetic analysis indicated that salacinol **3** or its analogues **A** could be obtained by alkylation of anhydro-alditol derivatives at the ring heteroatom (Scheme 1).[3] This strategy was chosen in order to provide flexibility for the synthesis of analogues having different heteroatoms (X) in the ring. We envisaged that the opening of appropriately protected cyclic sulfates with selenoether nucleophiles (X = Se) would likely proceed as well, or better, with the corresponding thioether derivatives.

The required seleno-anhydroarabinitol, 2,3,5-tri-*O*-benzyl-1,4-anhydro-4-seleno-D-arabinitol (**14**), was prepared from L-xylose as shown in Scheme 2. Thus, 2,3,5-tri-*O*-benzyl-L-xylitol (**12**) was synthesized in four steps starting from L-xylose, as described by Satoh et al.[10a] for the synthesis of its enantiomer. Mesylation

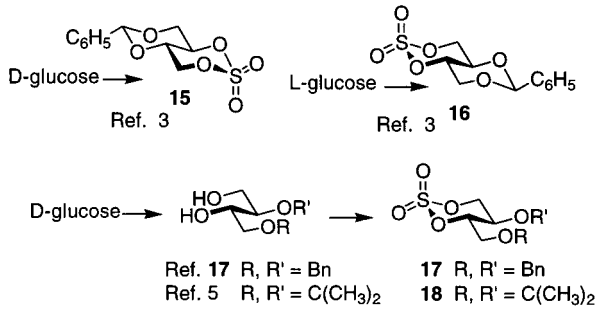
Scheme 1



Scheme 2



Scheme 3



produced the dimesylate **13**,[10b] which was reacted with freshly prepared Na_2Se to yield **14** in 80% yield (Scheme 2).

The 2,4-*O*-benzylidene-L- and D-erythritol-1,3-cyclic sulfates **15** and **16** were synthesized from L- and D-glucose, respectively, using our methods reported previously (Scheme 3).[3]

The selenonium salt **19** was synthesized by alkylation of the protected seleno-arabinitol **14** with the cyclic sulfate **16** (1.2 equiv) in dry acetone containing K_2CO_3, in excellent yield (86%) (Scheme 4), but NMR spectroscopy showed the presence of two isomers in a ratio of 7:1.

We have also investigated an alternative route to **19** which avoided the use of expensive L-glucose as a starting material. Yuasa et al.[5] have reported the preparation of the cyclic sulfates **17** and **18** from D-glucose (Scheme 3) and investigated their utility in the synthesis of salacinol. They concluded that the more reactive isopropylidene derivative **18** was the reagent of choice under their conditions and that **17** decomposed at temperatures of 60−70 °C in DMF. We also prepared **17** from D-glucose by a similar route (Scheme 3) and have confirmed that this derivative is a much less reactive alkylating agent than the corresponding benzylidene compound **15** that we had employed previously.[3] Thus, attempted reactions with **17** for the preparation of precursors to salacinol, under a variety of

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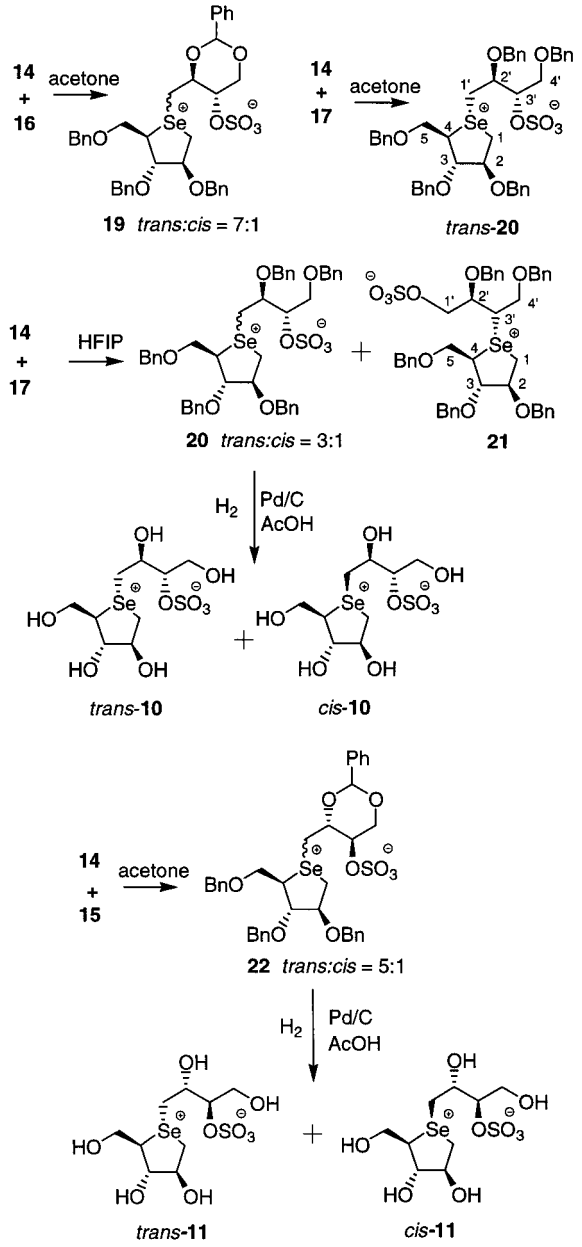
Scheme 4



different reaction conditions, were unsuccessful. However, the more nucleophilic selenium derivative **14** did lead, under our standard conditions, to a low yield of the selenonium salt **20**, which was obtained as a single diastereomer (Scheme 4). The reaction proceeded very slowly at 85 °C in acetone and was terminated before complete consumption of the selenoether because decomposition products were formed after extended periods. The NOESY spectrum of **20** showed a clear correlation between H-4 and H-1′, thus indicating the presence of the isomer with a trans relationship between C-5 and C-1′.

As part of a study of the influence of different solvents in such reactions, we investigated the effects of the unusual solvent 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP). This solvent has been shown to promote the nucleophilic ring-opening of epoxides by amines.[11] The beneficial effects were attributed to activation of epoxides through hydrogen bonding with the relatively acidic hexafluoro alcohol ($pK_a = 9.3$). Since cyclic sulfates are often considered to be synthetic equivalents of epoxides, we tested HFIP as a reaction medium for selenonium salt formation. The reaction of the selenoether **14** with the cyclic sulfate **17** in HFIP was much faster and proceeded essentially to completion in less than 1 day at 80 °C (Scheme 4). Two more polar products were formed and were separated by chromatography. The major product was obtained in 78% yield and proved to be compound **20**, isolated as a 3:1 mixture of diastereomers at the stereogenic selenium center. The major diastereomer in the mixture was identical to the selenonium salt, *trans*-**20**, obtained from the reaction in acetone. The minor *cis*-**20** diastereomer showed the expected H-1′/H-5 correlation in the NOESY spectrum. The other minor product, **21** (4%), was obtained isomerically pure and was assigned to be the product resulting from attack of the selenoether at the secondary carbon (C-3′) of the cyclic sulfate. This mode of attack at the more hindered center was not observed in the corresponding reaction of the thioether[3] and presumably arises because of the longer C−Se versus C−S bond, thereby leading to fewer steric interactions during Se−C bond formation, and also because of the affinity of the softer Se atom for the softer secondary carbon center. Alternatively, the acidic nature of HFIP could have resulted in an S_N1-type reaction. The ^{1}H NMR spectrum of **21**, compared to that of **20**, showed downfield shifts for the resonances of H-1′ and an upfield shift of the H-3′ resonance. Similar differences in the chemical shifts for C-1′ and C-3′ in the ^{13}C NMR spectrum provided confirmation of the interchange of selenonium and sulfate centers. Thus, although HFIP resulted in a faster reaction and a higher overall yield, the selectivity for the desired *trans*-**20** isomer had decreased.

Hydrogenolysis of the benzyl protecting groups of **20** (trans:cis = 3:1) yielded **10** as a mixture of diastereomers. Precipitation from MeOH yielded amorphous selenosalacinol (*trans*-**10**) of >90% diastereomeric purity at the stereogenic selenium center. We have chosen to name the selenium analogue of salacinol (*trans*-**10**) "blintol". The ^{1}H and ^{13}C NMR spectra of blintol (*trans*-**10**) were very similar to those of salacinol (**3**) except for slight upfield shifts for resonances corresponding to the nuclei in close proximity to the selenium center and the magnitudes of the coupling constants. As previously noted for salacinol itself,[3] these spectra were sensitive to concentration effects due to solvation of molecular aggregates of charged zwitterionic species.

Since we had previously shown that a salacinol analogue having the side chain derived from D-erythritol instead of L-erythritol showed some glycosidase inhibitory activity,[6] the corresponding selenium analogue **22** was prepared starting from the seleno-arabinitol **14** and the cyclic sulfate **15**. Reaction of these partners in acetone under our standard conditions yielded the selenonium salt **22** (78%) as a mixture of two diasteromers (5:1) at the stereogenic selenium center. The major component of the mixture was *trans*-**22**, having C-1′ and C-5 in a trans relationship. Deprotection of **22** was problematic. Essentially no hydrogenolysis occurred under 1 atm of H_2, despite several additions of more Pd/C catalyst. Eventually the starting material was isolated and repurified to remove catalyst-poisoning impurites. Deprotection by hydrogenolysis was then achieved, albeit in very low yield (19%), and the selenonium salt **11** was obtained as a crystalline solid (trans:cis, 8:1). We attribute the

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low yield to loss of the selenonium salt intermediates by adsorption on the large amounts of Pd/C used. Recrystallization of the diasteromeric mixture from MeOH gave pure *trans*-**11**.

Enzyme Inhibition Assays. Compounds *trans*-**10** and *trans*-**11** were tested for their inhibition of three glycosidase enzymes, namely glucoamylase G2,[12,13] porcine pancreatic α-amylase (PPA), and barley α-amylase (AMY1).[14] The effects were compared to those of salacinol (**3**). Glucoamylase G2 was weakly inhibited by **3** (K_i = 1.7 mM), whereas *trans*-**10** was a better inhibitor of this enzyme, with a K_i value of 0.72 mM. In contrast, *trans*-**11** showed no significant inhibition of glucoamylase. Salacinol inhibited AMY1 and PPA, with K_i values of 15 ± 1 and 10 ± 2 μM, respectively. Surprisingly, *trans*-**10** and *trans*-**11** showed no significant inhibition of either AMY1 or PPA. It would appear, then, that analogues *trans*-**10**, *trans*-**11**, and **3** show discrimination for certain glycosidase enzymes and could be promising candidates for selective inhibition of a wider panel of enzymes that includes human small intestinal maltase-glucoamylase[15] and human pancreatic α-amylase.[16] Such studies to map the enzyme selectivity profiles of these compounds are planned.

Experimental Section

General. Optical rotations were measured at 23 °C. ^{1}H and ^{13}C NMR spectra were recorded at 400.13 and 100.6 MHz, respectively, unless otherwise stated. All assignments were confirmed with the aid of two-dimensional ^{1}H, ^{1}H (COSYDFTP) or ^{1}H, ^{13}C (INVBTP) experiments using standard Bruker pulse programs. Column chromatography was performed with Merck silica gel 60 (230−400 mesh). MALDI-TOF mass spectra were measured on a PerSeptive Biosystems Voyager-DE spectrometer, using 2,5-dihydroxybenzoic acid as a matrix.

1,4-Anhydro-2,3,5-tri-*O*-benzyl-4-seleno-D-arabinitol (14). Selenium metal (1.1 g, 14 mmol) was added to liquid NH_3 (60 mL) in a −50 °C bath, and small pieces of sodium (0.71 g) were added until a blue color appeared. A small portion of selenium (20 mg) was added to remove the blue color. NH_3 was removed by warming on a water bath, and DMF was added and removed under high vacuum to remove the rest of the NH_3. A solution of the dimesylate (**13**) (7.4 g, 12.7 mmol) in DMF (100 mL) was added, and the mixture was stirred under N_2 in a 70 °C bath for 3 h. The mixture was cooled, and the solvent was removed under high vacuum. The product was partitioned between CH_2Cl_2 (150 mL) and water (50 mL), and the organic solution was washed with water (50 mL) and brine (50 mL) and dried ($MgSO_4$). The solvent was removed, and the product was purified by flash chromatography (hexanes:EtOAc, 3:1) to give **14** as a pale yellow oil (4.74 g, 80%). $[\alpha]_D$: +22° (*c* 1.3, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.48−7.22 (15H, m, Ar), 4.67, 4.61 (2H, 2d, $J_{A,B}$ = 11.8 Hz, C*H*$_2$Ph), 4.56, 4.48 (2H, 2d, $J_{A,B}$ = 12.1 Hz, C*H*$_2$Ph), 4.53, 4.50 (2H, 2d, C*H*$_2$Ph), 4.22 (1H, ddd, H-2), 4.07 (1H, dd, $J_{2,3}$ = $J_{3,4}$ = 4.6 Hz, H-3), 3.85 (1H, dd, $J_{5a,5b}$ = 9.2, $J_{4,5a}$ = 7.6 Hz, H-5a), 3.77 (1H, ddd, $J_{4,5b}$ = 6.9 Hz, H-4), 3.53 (1H, dd, H-5b), 3.11 (1H, dd, $J_{1a,1b}$ = 10.4, $J_{1a,2}$ = 5.1 Hz, H-1a), 2.96 (1H, dd, $J_{1b,2}$ = 5.3 Hz H-1b). ^{13}C NMR ($CDCl_3$): δ 138.24, 138.21, 138.06 (3C$_{ipso}$), 128.40−127.60 (15C, Ph), 85.93 (C-2), 85.63 (C-3), 72.96 (2C, C-5, *C*H$_2$Ph), 72.14, 71.50 (2 × *C*H$_2$Ph), 42.59 (C-4), 23.96 (C-1). MALDI MS: *m*/*e* 491.2 (M^+ + Na). Anal. Calcd for $C_{26}H_{28}O_3S$: C, 74.25; H, 6.71. Found: C, 74.18; H, 6.53.

2,4-Di-*O*-benzyl-L-erythritol-1,3-*O*-cyclic Sulfate (17). A solution of 1,3-di-*O*-benzyl-D-erythritol[17] (10.0 g, 33.1 mmol) in CH_2Cl_2 and Et_3N (15 mL, 108 mmol) was cooled and stirred in an ice bath. Thionyl chloride (2.6 mL, 36 mmol) in CH_2Cl_2 (20 mL) was added dropwise over 0.5 h. After an additional 5 min, the mixture was poured onto crushed ice (∼100 g), and the aqueous phase was separated and extracted with additional CH_2Cl_2. The combined extracts were washed with cold water and dried over $MgSO_4$. The solvent was removed to give a mixture of the two diastereomeric cyclic sulfites as a pale brown oil. The mixture was passed through a short silica gel column with hexanes:EtOAc 3:1, without attempting to separate the isomers, and the mixture of cyclic sulfites (a pale yellow oil, 10.5 g) was immediately dissolved in 1:1 CH_3CN:CCl_4 (200 mL). Sodium periodate (10.6 g, 49.6 mmol) and $RuCl_3$·*x*H_2O (150 mg) were added, and the mixture was stirred rapidly while H_2O (100 mL) was added. After 75 min, analysis by TLC showed formation of a single, slightly more polar product. The mixture was poured into a separatory funnel, and the lower organic phase was separated. The dark red-brown aqueous phase was extracted with additional CCl_4, and the combined extracts were filtered and concentrated to a dark syrup that was dissolved in EtOAc (200 mL) and filtered to remove black material. The colorless filtrate was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl and dried over $MgSO_4$. Solvent removal gave an oil which was purified by flash chromatography (hexanes:EtOAc, 3:1) to yield **17** as a colorless syrup (9.66 g, 80%). Storage at −20 °C resulted in slow crystallization. A sample was recrystallized from Et_2O/hexanes. Mp: 63−65 °C. $[\alpha]^{20}_D$: −9.4° (*c* 1.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.40−7.20 (m, 10H, Ph), 4.76 (ddd, 1H, $J_{2,3}$ = 9.4, $J_{3,4a}$ = 3.3, $J_{3,4b}$ = 2.1 Hz, H-3), 4.64 and 4.53 (2d, each 1H, $J_{A,B}$ = 11.9 Hz, C*H*$_2$Ph), 4.59 and 4.50 (2d, each 1H, $J_{A,B}$ = 11.6 Hz, C*H*$_2$Ph), 4.44 (dd, 1H, $J_{1ax,1eq}$ = 11.0, $J_{1ax,2}$ = 10.1 Hz, H-1ax), 4.33 (dd, 1H, $J_{1eq,2}$ = 5.2 Hz, H-1eq), 4.17 (ddd, 1H, H-2), 3.88 (dd, 1H, $J_{4a,4b}$ = 12.0 Hz, H-4a), 3.76 (dd, 1H, H-4b). ^{13}C NMR ($CDCl_3$): δ 137.19 and 136.53, (2 × C_{ipso}, 2 × Ph), 128.75, 128.65, 128.56, 128.14, 128.07 and 127.90 (each 2C, C_{ortho}, C_{meta}, C_{para}, 2 × Ph), 84.99 (C-3), 73.69 and 73.64 (2 × *C*H$_2$Ph), 71.59 (C-2), 66.92 and 66.50 (C-1, C-4). MALDI MS: *m*/*e* 387.2 (M^+ + Na), 403.1 (M^+ + K). Anal. Calcd for $C_{18}H_{20}O_6S$: C, 59.33; H, 5.53. Found: C, 59.38; H, 5.52.

2,3,5-Tri-*O*-benzyl-1,4-dideoxy-1,4-[[(2*S*,3*S*)-2,4-benzylidenedioxy-3-(sulfoxy)butyl]episelenoniumylidene]-D-arabinitol Inner Salt (19). The selenoether **14** (0.20 g, 0.43 mmol) was reacted with the cyclic sulfate **15** (0.14 g, 1.2 equiv) in acetone (0.7 mL) by the procedure used previously for the corresponding sulfide.[3] Column chromatography ($CHCl_3$:MeOH, 15:1) of the crude product gave an amorphous solid (0.27 g, 86%). NMR showed the presence of two isomers (7:1) at the stereogenic selenium center which were separable on analytical HPLC (acetonitrile/H_2O).

Data for the major diastereomer *trans*-**19** follow. ^{1}H NMR (CD_2Cl_2): δ 7.50−7.10 (20H, m, Ar), 5.55 (1H, s, C*H*Ph), 4.58 (1H, m, H-2), 4.56−4.45 (5H, m, H-3′, 3 C*H*$_2$Ph, H-4′eq), 4.38 (1H, dd, $J_{1'a,2'}$ = 2.2 Hz, H-1′a), 4.38−4.34 (2H, m, H-3, C*H*$_2$Ph), 4.34 and 4.26 (2H, 2d, $J_{A,B}$ = 12.1 Hz, C*H*$_2$Ph), 4.25 (1H, m, H-2′), 4.14−4.08 (2H, m, H-1a, H-4), 3.97 (1H, dd, $J_{1'a,1'b}$ = 12.1, $J_{1'b,2'}$ = 3.3 Hz, H-1′b), 3.80 (1H, dd, $J_{4'ax,4'eq}$ = $J_{3',4'ax}$ = 10.1 Hz, H-4′ax), 3.65−3.52 (3H, m, H-1b, H-5a, H-5b). ^{13}C NMR (CD_2Cl_2): δ 137.28−126.68 (24C$_{Ar}$), 102.10 (*C*HPh), 84.55 (C-3), 83.36 (C-2), 77.18 (C-2′), 73.70, 72.81, 72.36 (3 × *C*H$_2$Ph), 69.57 (C-4′), 67.76 (C-3′), 67.02 (C-5), 66.30 (C-4), 48.77 (C-1′), 46.43 (C-1). Anal. Calcd for $C_{37}H_{40}O_9SSe$: C, 59.99; H, 5.45. Found: C, 59.91; H, 5.44.

2,3,5-Tri-*O*-benzyl-1,4-dideoxy-1,4-[[(2*S*,3*S*)-2,4-di(benzyloxy)-3-(sulfoxy)butyl]episelenoniumylidene]-D-arabinitol Inner Salt (20). (a) By Reaction in $(CH_3)_2CO$. The selenoether **14** (117 mg, 0.250 mmol), cyclic sulfate **17** (84 mg, 0.23 mmol), and K_2CO_3 (80 mg, 0.58

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mmol) were added to anhydrous acetone (3.0 mL), and the mixture was stirred in a sealed tube with heating at 70 °C for 20 h and then at 85 °C for 48 h. Periodic analysis by TLC ($CHCl_3$:MeOH, 10:1) showed that the reaction was proceeding very slowly and that substantial fractions of the cyclic sulfate **17** and the selenoether **14** remained unreacted. Slow decomposition of the cyclic sulfate to produce polar impurities was noted at the higher temperature. At the end of 68 h, the extent of reaction to yield the desired product was estimated to be <30%, but decomposition products were becoming significant, and thus, the reaction was terminated at this point. The mixture was cooled and filtered through Celite with the aid of CH_2Cl_2. The solvents were removed, and the residue was purified by column chromatography (gradient of $CHCl_3$ to $CHCl_3$:MeOH, 10:1). The selenonium salt **20** (39 mg, 20%) was obtained as a colorless syrup. Compound **20** was isomerically pure and was assigned to be the isomer with a trans relationship between C-5 and C-1′ by analysis of the NOESY spectrum.

Data for *trans*-**20** follow. $[\alpha]_D$: −15° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.38−7.05 (25H, m, Ph), 4.65 and 4.46 (2H, 2d, $J_{A,B}$ = 12.0 Hz, C*H*$_2$Ph), 4.64 (1H, dt, $J_{2',3'}$ = 6.9 Hz, H-3′), 4.63 and 4.47 (2H, 2d, $J_{A,B}$ = 11.6 Hz, C*H*$_2$Ph), 4.48 and 4.33 (2H, 2d, $J_{A,B}$ = 11.8 Hz, C*H*$_2$Ph), 4.46 (1H, dd, H-2), 4.43 and 4.38 (2H, 2d, $J_{A,B}$ = 11.2 Hz, C*H*$_2$Ph), 4.38 and 4.28 (2H, 2d, $J_{A,B}$ = 12.0 Hz, C*H*$_2$Ph), 4.28 (1H, ddd, H-2′), 4.24 (1H, br d, $J_{2,3}$ = 1.6 Hz, H-3), 4.22 (1H, br d, H-1a), 4.17 (1H, dd, $J_{1'a,1'b}$ = 12.1, $J_{1'a,2'}$ = 1.1 Hz, H-1′a), 4.04 (1H, dd, $J_{4'a,4'b}$ = 11.1, $J_{3',4'a}$ = 2.9 Hz, H-4′a), 3.87 (1H, dd, $J_{3',4'b}$ = 2.5 Hz, H-4′b), 4.50 (1H, br t, H-4), 3.67 (1H, dd, $J_{1'b,2'}$ = 4.3 Hz, H-1′b), 3.60 (1H, dd, $J_{1a,1b}$ = 12.8, $J_{1b,2}$ = 3.0 Hz, H-1b), 3.57 (1H, dd, $J_{5a,5b}$ = 10.1, $J_{4,5a}$ = 7.1 Hz, H-5a), 3.54 (1H, dd, $J_{4,5b}$ = 9.0 Hz, H-5b). ^{13}C NMR ($CDCl_3$): δ 137.07, 137.00, 136.87, 136.15 and 135.88 (5 × C_{ipso}, Ph), 128.81−127.60 (25C, Ph), 83.83 (C-3), 81.16 (C-2), 74.99 (C-3′), 73.79 and 73.40 (2 × *C*H$_2$Ph), 75.18 (C-2′), 72.85, 72.01, and 71.59 (3 × *C*H$_2$Ph), 69.14 (C-4′), 67.13 (C-5), 64.83 (C-4), 50.08 (C-1′), 46.34 (C-1). MALDI MS: *m*/*e* 833.8 (M^+ + H), 753.7 (M^+ + H − SO_3). Anal. Calcd for $C_{44}H_{48}O_9SSe$: C, 63.53; H, 5.82. Found: C, 63.39; H, 5.86.

(b) By Reaction in $(CF_3)_2$CHOH. The selenoether **14** (633 mg, 1.35 mmol), cyclic sulfate **17** (531 mg, 1.46 mmol), and K_2CO_3 (73 mg, 0.53 mmol) were added to 1,1,1,3,3,3-hexafluoro-2-propanol (2.0 mL), and the mixture was stirred in a Caries tube while being warmed slowly. The K_2CO_3 dissolved with evolution of gas between 60 and 80 °C. The tube was cooled to room temperature, opened to relieve pressure, and then resealed and kept at 80 °C for 22 h. Analysis by TLC ($CHCl_3$:MeOH, 10:1) showed virtually complete consumption of the cyclic sulfate **17**, although some of the selenoether **14** remained unreacted. Two products of increased polarity had been formed (major, rf 0.40; minor, rf 0.35). The mixture was cooled and filtered through Celite with the aid of CH_2Cl_2. The solvents were removed, and the residue was purified by column chromatography (two successive silica gel columns: first with a gradient of $CHCl_3$ to $CHCl_3$:MeOH, 10:1, to separate the starting materials, and then with EtOAc:MeOH, 25:1, to separate the two products). The selenonium salts **20** (827 mg, 78%) and **21** (45 mg, 4%) were obtained as syrupy oils. Compound **20** proved to be a 3:1 mixture of isomers at the stereogenic selenium center. The major component (*trans*-**20**) was identical to the compound obtained from the reaction in acetone (that is, the trans C-5, C-1′ isomer), while the minor component was assigned to be the corresponding *cis*-**20** isomer. Partial ^{13}C NMR data for the cis isomer were obtained from a spectrum of the mixture, and assignments were made by analogy to those of the trans isomer. Compound **21** was isomerically pure and was assigned to be the isomer with a trans relationship between C-5 and C-3′ by analysis of the NOESY spectrum.

Data for the *cis*-**20** isomer follow. ^{13}C NMR ($CDCl_3$): 84.31 (C-3), 82.78 (C-2), 75.42 (C-3′), 73.84 and 73.52, (2 × *C*H$_2$Ph), 73.18 (C-2′), 72.86 (*C*H$_2$Ph), 71.72 (2 × *C*H$_2$Ph), 68.78(C-4′), 65.46 (C-5), 58.33 (C-4), 42.71 (C-1′), 40.16 (C-1).

Data for **21** follow. $[\alpha]^{20}_D$: −68° (*c* 2.2, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.38−7.00 (25H, m, Ph), 4.76 (1H, dt, $J_{2',3'}$ = 7.0 Hz, H-3′), 4.71 and 4.42 (2H, 2d, $J_{A,B}$ = 10.9 Hz, C*H*$_2$Ph), 4.68 and 4.51 (2H, 2d, $J_{A,B}$ = 12.4 Hz, C*H*$_2$Ph), 4.50 and 4.36 (2H, 2d, $J_{A,B}$ = 11.6 Hz, C*H*$_2$Ph), 4.50 (1H, ddd, H-4), 4.38 (1H, dd, $J_{1'a,1'b}$ = 12.6, $J_{1'a,2'}$ = 5.1 Hz, H-1′a), 4.36−4.32 (2H, m, H-2, H-3), 4.32 and 4.14 (2H, 2d, $J_{A,B}$ = 11.7 Hz, C*H*$_2$Ph), 4.29 (1H, dd, $J_{1'b,2'}$ = 3.1 Hz, H-1′b), 4.28 and 4.21 (2H, 2d, $J_{A,B}$ = 11.8 Hz, C*H*$_2$Ph), 4.13 (1H, ddd, H-2′), 4.10 (1H, dd, $J_{4'a,4'b}$ = 11.8, $J_{3',4'a}$ = 3.8 Hz, H-4′a), 3.84 (1H, dd, $J_{3',4'b}$ = 3.8 Hz, H-4′b), 3.48 (1H, dd, $J_{5a,5b}$ = 10.0, $J_{4,5a}$ = 9.1 Hz, H-5a), 3.44 (1H, dd, $J_{1a,1b}$ = 12.0 Hz, H-1a), 3.42 (1H, dd, $J_{4,5b}$ = 9.9 Hz, H-5b), 3.14 (1H, dd, $J_{1b,2}$ = 2.2 Hz, H-1b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.15, 136.63, 136.57, 136.34 and 136.11 (5 × C_{ipso}, Ph), 128.89−127.50 (25C, Ph), 83.43 (C-3), 81.12 (C-2), 75.35 (C-2′), 73.55 (C-1′), 73.06, 72.40, 71.85 and 71.65 (4 × *C*H$_2$Ph), 66.31 (C-5), 65.90 (C-4′), 63.79 (*C*H$_2$Ph), 62.65 (C-4), 61.73 (C-3′), 39.77 (C-1). MALDI MS: *m*/*e* 833.8 (M^+ + H), 753.8 (M^+ + H − SO_3). Anal. Calcd for $C_{44}H_{48}O_9SSe$: C, 63.53; H, 5.82. Found: C, 63.79; H, 5.83.

2,3,5-Tri-*O*-benzyl-1,4-dideoxy-1,4-[[(2*R*,3*R*)-2,4-benzylidenedioxy-3-(sulfoxy)butyl]episelenoniumylidene]-D-arabinitol Inner Salt (22). The selenoether **14** (760 mg, 1.63 mmol), cyclic sulfate **16**[3] (467 mg, 1.72 mmol), and K_2CO_3 (102 mg, 0.74 mmol) were added to anhydrous acetone (3.0 mL), and the mixture was stirred in a sealed tube with heating at 80 °C for 13 h. Analysis by TLC ($CHCl_3$:MeOH, 10:1) showed that the cyclic sulfate **16** had been consumed but that there was a substantial amount of the selenoether **14** remaining. Another portion of the cyclic sulfate (180 mg, 0.66 mmol) was therefore added, and the reaction was continued at 80 °C for a further 12 h. After cooling to room temperature, the mixture was diluted with CH_2Cl_2 and processed and purified as described for **19**. Compound **22** was obtained as a colorless foam (0.939 g, 78%). Analysis by NMR showed that the product was a mixture of two isomers (~5:1) at the stereogenic selenium center. The major component of the mixture was assigned to be the diastereomer with a trans relationship between C-5 and C-1′ on the basis of analysis of the NOESY spectrum.

Data for the major diastereomer (*trans*-**22**) follow. ^{1}H NMR (CD_2Cl_2): δ 7.50−7.10 (25H, m, Ph), 5.54 (1H, s, C*H*Ph), 4.58 and 4.50 (2H, 2d, $J_{A,B}$ = 12.0 Hz, C*H*$_2$Ph), 4.55 (1H, dd, $J_{4'ax,4'eq}$ = 10.6, $J_{3',4'eq}$ = 5.1 Hz, H-4′eq), 4.50 (1H, dd, H-2), 4.45 (1H, br d, $J_{2,3}$ = 2.6 Hz, H-3), 4.44 (1H, ddd, H-3′), 4.40 and 4.35 (2H, 2d, $J_{A,B}$ = 11.7 Hz, C*H*$_2$Ph), 4.35 and 4.23 (2H, 2d, $J_{A,B}$ = 11.9 Hz, C*H*$_2$Ph), 4.34 (1H, ddd, H-2′), 4.33 (1H, br t, H-4), 4.16 (2H, br d, $J_{1',2'}$ = 4.9 Hz, H-1′a, H-1′b), 3.91 (1H, dd, $J_{1a,2}$ = 1.5, $J_{1a,1b}$ = 12.1 Hz, H-1a), 3.78 (1H, dd, $J_{3',4'ax}$ = 9.8 Hz, H-4′ax), 3.67−3.59 (2H, m, H-5a, H-5b), 3.56 (1H, dd, $J_{1b,2}$ = 3.4 Hz, H-1b). ^{13}C NMR (CD_2Cl_2): δ 137.66, 137.31, 136.72 and 136.49 (4 × C_{ipso}, Ph), 129.73−126.66 (25C, Ph), 102.04 (*C*HPh), 84.27 (C-2), 83.04 (C-3), 77.04 (C-2′), 73.60, 72.51 and 72.14 (3 × *C*H$_2$Ph), 69.77 (C-4′), 68.82 (C-3′), 67.05 (C-5), 64.81 (C-4), 48.19 (C-1′), 46.35 (C-1). MALDI MS: *m*/*e* 741.6 (M^+ + H), 661.5 (M^+ + H − SO_3). Anal. Calcd for $C_{37}H_{40}O_9SSe$: C, 60.08; H, 5.45. Found: C, 59.91; H, 5.45.

1,4-Dideoxy-1,4-[[(2*S*,3*S*)-2,4-dihydroxy-3-(sulfoxy)butyl]episelenoniumylidene]-D-arabinitol Inner Salt (10). To a solution of selenonium salt **20** (744 mg, 0.894 mmol, 3:1 mixture of isomers) in HOAc (10 mL) was added 10% Pd/C catalyst (200 mg), and the mixture was stirred under an atmosphere of H_2 for 16 h. More Pd/C (200 mg) was added, and the hydrogenolysis was continued for an additional 24 h. The mixture was filtered through Celite with MeOH (80 mL) and concentrated to give a syrup. Purification by column chromatography (EtOAc:MeOH:H_2O, 6:3:1) gave **10** as a colorless gum (225 mg, 66%). Analysis by ^{1}H NMR indicated a mixture of isomers (5:1). The product was dissolved in a minimum amount of warm MeOH and cooled slowly to deposit an amorphous solid (112 mg). This proved to be >90% pure **10** which was assigned, by analysis of the NOESY spectrum, to be the major *trans*-**10** isomer corresponding to the configuration of salacinol.

Data for the diastereomer *trans*-**10** follow. $[\alpha]^{20}_D$: +20° (*c* 0.5, D_2O). ^1H NMR (600 MHz, D_2O): δ 4.84 (1H, ddd, H-2), 4.53 (1H, dd, $J_{2,3}$ = 3.5 Hz, H-3), 4.43 (1H, ddd, $J_{2',3'}$ = 7.0 Hz, H-2′), 4.37 (1H, ddd, H-3′), 4.22 (1H, ddd, $J_{3,4}$ = 3.2 Hz, H-4), 4.12 (1H, dd, $J_{4,5a}$ = 5.1, $J_{5a,5b}$ = 12.5 Hz, H-5a), 4.04 (1H, dd, $J_{1'a,2'}$ = 3.7, $J_{1'a,1'b}$ = 12.4 Hz, H-1′a), 3.98 (1H, dd, $J_{4,5b}$ = 8.9 Hz, H-5b), 3.97 (1H, dd, $J_{3',4'a}$ = 3.6, $J_{4'a,4'b}$ = 12.8 Hz, H-4′a), 3.90 (1H, dd, $J_{1'b,2'}$ = 7.5 Hz, H-1′b), 3.88 (1H, dd, $J_{3',4'b}$ = 3.4 Hz, H-4′b), 3.86 (1H, dd, $J_{1a,2}$ = 3.2 Hz, H-1a), 3.83 (1H, dd, $J_{1b,2}$ = 4.0, $J_{1a,1b}$ = 12.2 Hz, H-1b). ^{13}C NMR (100 MHz, D_2O): δ 83.10 (C-2′), 80.98 (C-3), 80.27 (C-2), 72.58 (C-4), 68.32 (C-2′), 62.34 (C-4′), 61.93 (C-5), 50.29 (C-1′), 47.75 (C-1). MALDI MS: *m*/*e* 383.2 (M^+ + H), 303.2 (M^+ + H − SO_3). Anal. Calcd for $C_6H_{18}O_9SSe$: C, 28.35; H, 4.76. Found: C, 28.12; H, 4.83.

1,4-Dideoxy-1,4-[[(2*R*,3*R*)-2,4-dihydroxy-3-(sulfoxy)butyl]episelenoniumylidene]-D-arabinitol Inner Salt (11). Hydrogenolysis of the selenonium salt **22** (0.906 g, 1.22 mmol, trans:cis = 5:1) by the same procedure reported above for **19** was extremely sluggish. After a total of 1.2 g of Pd/C had been added in three portions over 3 days of stirring under an atmosphere of H_2, little reaction had occurred. The reaction was stopped and the catalyst removed by filtration through Celite with methanol. The solvents were removed, and the residue, consisting mostly of unreacted starting material **22**, was repurified by column chromatography. The fractions containing **22** were combined and concentrated to a syrup. Hydrogenolysis of this material in acetic acid (6 mL) with Pd/C (0.4 g) was now relatively rapid, and TLC indicated complete reaction after 24 h. Processing and purification as described for **10** gave **11** as a colorless foam (88 mg, 19%). This low yield was attributed to losses of the selenonium salt by adsorption on the large amounts of Pd/C that had been used. Analysis by ^1H NMR indicated that **11** was a mixture of isomers (8:1) at the selenium center. The major component of the mixture was assigned to be the diastereomer with a trans relationship between C-5 and C-1′ on the basis of observation of a strong H-1′/H-4 correlation in the NOESY spectrum. The pure *trans*-**11** was obtained by crystallization from MeOH.

Data for the major isomer *trans*-**11** follow. Mp: 137−140 °C. $[\alpha]^{20}_D$: −33° (*c* 0.3, H_2O). ^1H NMR (600 MHz, D_2O): δ 4.83 (1H, ddd, H-2), 4.54 (1H, dd, $J_{2,3}$ = 3.6 Hz, H-3), 4.43 (1H, td, $J_{1'a,2'}$ = $J_{2',3'}$ = 6.9, $J_{1'b,2'}$ = 5.5 Hz, H-2′), 4.36 (1H, ddd, H-3′), 4.28 (1H, ddd, $J_{3,4}$ = 3.2 Hz, H-4), 4.10 (1H, dd, $J_{4,5a}$ = 5.3, $J_{5a,5b}$ = 12.7 Hz, H-5a), 4.00 (1H, dd, $J_{4,5b}$ = 8.0 Hz, H-5b), 3.98 (1H, dd, $J_{3',4'a}$ = 3.1 Hz, H-4′a), 3.96 (2H, m, H-1′a, H-1′b), 3.87 (1H, dd, $J_{3',4'b}$ = 3.4, $J_{4'a,4'b}$ = 12.8 Hz, H-4′b), 3.82 (2H, d, $J_{1a,2}$ = $J_{1b,2}$ = 4.0 Hz, H-1a, H-1b). ^{13}C NMR (D_2O): δ 83.33 (C-3′), 81.15 (C-3), 80.34 (C-2), 75.54 (C-4), 68.67 (C-2′), 62.36 (C-4′), 61.86 (C-5), 49.96 (C-1′), 47.30 (C-1). MALDI MS: *m*/*e* 383.0 (M^+ + H), 303.0 (M^+ + H − SO_3). Anal. Calcd for $C_6H_{18}O_9SSe$: C, 28.35; H, 4.76. Found: C, 28.30; H, 5.01.

Enzyme Inhibition Assays. The glucoamylase G2 form from *Aspergillus niger* was purified from a commercial enzyme (Novo Nordisk, Bagsvaerd, Denmark) as described.[12,13] The initial rates of glucoamylase G2-catalyzed hydrolysis of maltose were tested with 1 mM maltose as substrate in 0.1 M sodium acetate pH 4.5 at 45 °C using an enzyme concentration of 7.0×10^{-8} M and five inhibitor concentrations in the range from 1 μm to 5 mM. The effects of the inhibition on rates of substrate hydrolysis were compared for the different compounds. The glucose released was analyzed in aliquots removed at appropriate time intervals using a glucose oxidase assay adapted to microtiter plate reading and using a total reaction volume for the enzyme reaction mixtures of 150 or 300 μL.[18] The K_i values were calculated assuming competitive inhibition from $1/v = (1/V_{max}) + [(K_m)/(V_{max}[S]K_i)][I]$, where v is the rate measured in the presence or absence of inhibitor, [I] and [S] the concentrations of inhibitor and substrate, K_m = 1.6 mM, and k_{cat} = 11.3 s^{-1}, using ENZFITTER.[19]

Porcine pancreatic α-amylase (PPA) and bovine serum albumin (BSA) were purchased from Sigma. Amylose EX-1 (DP17; average degree of polymerization 17) was purchased from Hayashibara Chemical Laboratories (Okayama, Japan). Recombinant barley α-amylase isozyme 1 (AMY1) was produced and purified as described.[14] An aliquot of the porcine pancreatic α-amylase (PPA) crystalline suspension (in ammonium sulfate) was dialyzed extensively against the assay buffer without BSA. The enzyme concentration was determined by aid of amino acid analysis as determined using an LKB model Alpha Plus amino acid analyzer. The inhibition of AMY1 (3×10^{-9} M) and PPA (9×10^{-9} M) activity toward DP17 amylose was measured at 37 °C in 20 mM sodium acetate, pH 5.5, 5 mM $CaCl_2$, 0.005% BSA (for AMY1), and 20 mM sodium phosphate, pH 6.9, 10 mM NaCl, 0.1 mM $CaCl_2$, 0.005% BSA (for PPA). Six different final inhibitor concentrations were used in the range from 1 μM to 5 mM. The inhibitor was preincubated with enzyme for 5 min at 37 °C before addition of substrate. Initial rates were determined by measuring reducing sugar by the copper−bicinchoninate method as described.[14,20] The K_i values were calculated assuming competitive inhibition, as described above for the case of glucoamylase, and a K_m of 0.57 mg/mL and k_{cat} of 165 s^{-1} for AMY1 and 1 mg/mL and 1200 s^{-1}, respectively, for PPA, as determined in the substrate concentration range 0.03−10 mg/mL using ENZFITTER.[19] For the K_i determinations, [S] = 0.7 mg/mL amylose DP 17 for the AMY1 binding and [S] = 2.5 mg/mL amylose DP 17 for the PPA binding.

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