JOC Article

The Synthesis and Preliminary Biological Evaluation of a Novel Steroid with Neurotrophic Activity: NGA0187

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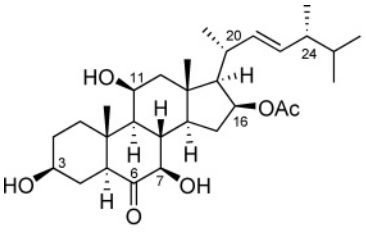
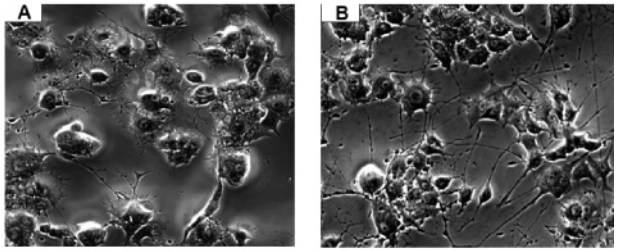


NGA0187 (**1**)



Images of neurons after treatment with (A) DMSO + NGF, (B) NGA0187 in DMSO (30 μM) + NGF.

A full account of the total synthesis of neurotrophic compound NGA0187 is provided. A key feature of the synthesis involved the direct selective oxidation of 6α,7β-diol to install the unusual 6,7-ketol moiety and stereoselective conjugate addition of vinyl cuprate to enone. A preliminary evaluation of its ability to stimulate the neurite outgrowth was also evaluated with PC12 cell.

Introduction

A pressing contemporary medical challenge is that of finding effective means for the treatment of neurodegenerative failures such as are found in Alzheimer's, Parkinson's, Huntington's, and Lou Gehrig's diseases. Nerve cells die by murder or suicide through programmed cell death (apoptosis)[1a] and require trophic factors to survive. The best characterized neurotrophic factors, or neurotrophins (cf. NGF, BDNF, NT-3, GDNF, NTN, ART, PSP, NT-4, -5, -6), are naturally occurring polypeptides or proteins which can prevent neuronal death (neurotrophism) or even promote axonal growth (neurotropism) after injury.[1b] Because of the enormous interest in neurotrophins since NGF was first discovered 50 years ago by Levi-Montalcini and Hamburger,[1c,d] neurotrophic factors have been the focus of interdisciplinary research for their potential in the treatment of neurologically centered disorders. Peptidyl neurotrophic factors have been tested in the context of neurodegenerative diseases. However, to date, none has been demonstrably effective, presumably because of the poor pharmacokinetic profiles exhibited by such compounds, resulting in marginal stability and inadequate delivery. In principle, the field could gain significantly from the discovery and development of nonpeptidic[1e] CNS-permeable molecules with neurotrophic or neurotropic activity.

The goal of evaluating putative small molecule neurotrophic factors has been of great interest in our laboratory in the past few years. Our approach, as per custom, focuses on natural product leads for which various claims of neurotrophic activity have been registered. Our discovery progression sets as its first milestone, the achievement of access to these natural products through the medium of total chemical synthesis. With the natural product in hand, we can investigate analogues synthetically derivable from the target system and can begin to build an SAR profile. Moreover, of increasing importance are the potentialities inherent in diverted

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(1) (a) Lodish, H.; Berk, A.; Zipursky, S. L.; Matsudaria, P.; Baltimore, D.; Darnell, J. *Molecular Cell Biology*, 4th ed.; W. H. Freeman & Co., New York: Section 23.8, 1999. (b) Kaneko, M.; Saito, Y.; Saito, H.; Matsumoto, T.; Matsuda, Y.; Vaught, J. L.; Dionne, C. A.; Angeles, T. S.; Glicksman, M. A.; Neff, N. T.; Rotella, D. P.; Kauer, J. C.; Mallamo, J. P.; Hudkins, R. L.; Murakata, C. *J. Med. Chem.* **1997**, *40*, 1863−1869. (c) Levi-Montalcini, R.; Hamburger, V. *J. Exp. Zool.* **1951**, *116*, 321−362. (d) Bennett, M. R.; Gibson, W. G.; Lemon, G. *Auton. Neurosci.* **2002**, *95*, 1−23. (e) Hefti, F. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 255−257.

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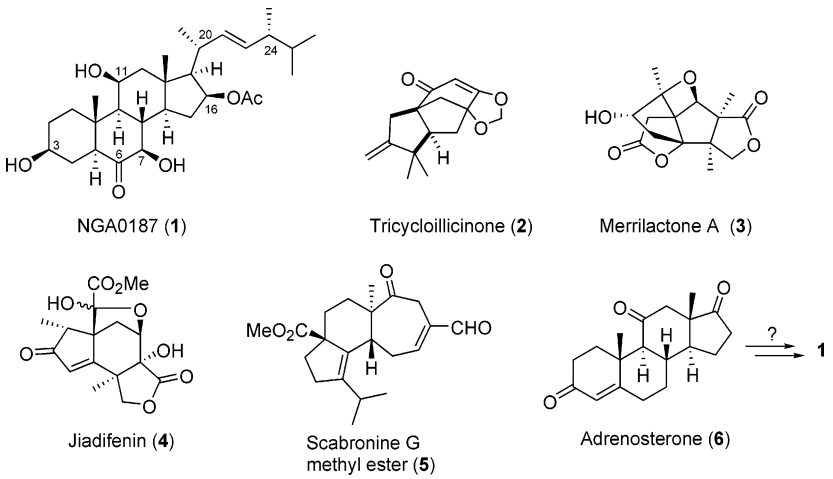

FIGURE 1. Structure of natural product neurotrophic factors.

total synthesis. By interdicting the natural product focused total synthesis before its conclusion, we are in a position to explore valuable chemical space which might not be available from the target system itself. Our earlier targets have been terpenoid compounds with claimed neurotrophic activities. At this early stage of the work, we do not necessarily insist on a defined molecular target for initiation of the synthetic program. Assuming that useful and promising activity can be corroborated and optimized, it is expected that one or more molecular targets will in time be identified.

Previous to the effort documented herein, our laboratory had accomplished the total syntheses of tricycloillicinone (**2**),[2a] merrilactone A (**3**),[2b,c] jiadifenin (**4**),[2d] and scabronine G methyl ester (**5**)[2e] (Figure 1). Recently we have initiated collaborative studies to learn about the consequences and mechanisms of action of these small molecule neurotrophic factors. Given our current lack of insight at the mechanistic level, the possibilities for failure of any particular initiative are not improbable. Nonetheless, in the light of their records in a range of whole cell functional assays, such molecules might represent major opportunities at the interface of chemistry and the neurosciences. In short, the conditions for admission to our total synthesis-enabled, admittedly elitist, library are (i) promising functional properties and (ii) a natural products lineage.

The focusing target of our research herein was a molecule termed NGA0187 (**1**). This compound was isolated, and its structure was determined by Nozawa et al. (via X-ray crystallography), in the course of a neurotrophically based screening program directed to the microorganism *Acremonium sp.* TF-0356.[3] The neurite outgrowth and cell survival activities of NGA0187 were reported by her research group. Although compound **1** showed little ability to protect rat cerebral cortical neuronal cells against damage, it did significantly induce neurite outgrowth, at doses of 30 μg/mL, corresponding to NGF at 10 ng/mL.[3]

To the best of our knowledge, NGA0187 is the first discovered steroid with significant neurotrophic activity. Furthermore, compound **1** struck us as bearing a rough homology with compounds **2**−**5** in its dense confluence of polar functions all residing on the same β-face at carbons 3,7,11,16. The ketone at C_6 provides additional polar functionality in the core sector. Thus, we came to look upon compound **1** in the context of a stretched out version of the more compacted structures **2**−**5**. Accordingly, we set out to accomplish a total synthesis of compound **1**. As matters transpired, this turned out to be a nontrivial goal, but with considerable persistence, it was accomplished.

Results and Discussions

Overall Synthetic Strategy. The main hurdles to overcome in a synthesis of NGA0187 were seen to include (i) the stereoselective introduction of the side chain at C_{17} with provision for the proper stereochemistry at the C_{24}, (ii) accommodation of the 16-acetoxyl function in the context of the C_3, C_7, and C_{11} hydroxyl groups, (iii) introduction and maintenance of the mutual 6-keto-7β-hydroxy subunit, and (iv) the proper provision for the 11-hydroxy group. While none of these implementations appeared to be unmanageable, their accommodation in the face of interactivity issues presented significant problems of functional group orchestration.

Unlike the terpenoid neurotrophins **2**−**5** wherein the synthesis of each presented uncertainties at the level of skeletal architecture, the primary challenge to a synthesis of compound **1** was that of functional group manipulation. As will be seen in the synthesis of NGA0187, the management of this type of problem, requiring exploitation of what are often subtle reactivity differentials, may be daunting in its own way.

Considerable thought was given to selection of a suitable starting material. Of course, we would begin with a steroid. However, several possibilities still present themselves in this context. Following careful study of the issues and the precedents, it was decided that it would be easier to introduce the entire steroid side chain (C_{20} onward) by attachment to C_{17} than to deal with introduc-

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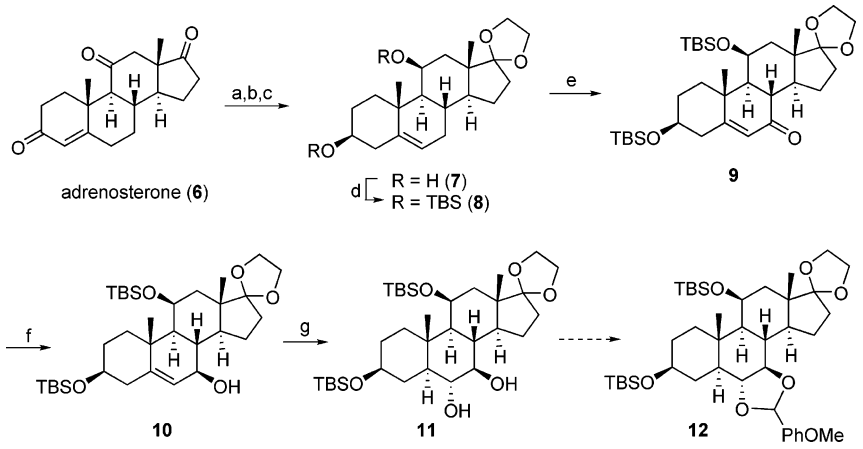
SCHEME 1. Synthesis of 6α,7β-diol Steroid 11 and Attempted Acetalization[a]

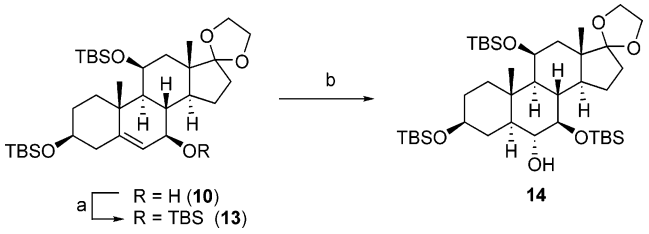


[a] Conditions: (a) AcCl, Ac_2O, reflux, 17 h; (b) $HC(OMe)_3$, $(CH_2OH)_2$, *p*-TsOH·H_2O, CH_2Cl_2, reflux, 1.5 h; (c) $NaBH_4$, ^{t}BuOH-THF-H_2O, 0 °C to room temperature, 48 h, 73% (3 steps); (d) TBSOTf, 2,6-lutidine, CH_2Cl_2, reflux, 12 h, 84%; (e) CrO_3, 3,5-dimethylpyrazole, CH_2Cl_2, −25 °C, 4 h, 51%; (f) $NaBH_4$, $CeCl_3$·$7H_2O$, THF-MeOH, −40 °C, 1 h, 98%; (g) BH_3-THF, THF, 0 °C to room temperature, 12 h; NaOH, H_2O_2, 2 h, rt, 81%.

tion of the $C_{22,23}$-double bond, the C-methyl at C_{24}, and the oxygen at C_{16} from a starting material which already contained some part of the side chain. Hence, our starting steroid would contain simply a C_{17} keto function. Similarly, we thought it more straightforward to engineer the novel C_6-keto-C_7-β hydroxy diad de novo than to exploit available steroids with existing but nonoptimal handles already in the B-ring. Hence, we would settle for a Δ4−3 keto moiety in our precursor. In the case of C_{11}, it was proposed that it would actually be easier to sustain a preexisting oxygen function at this carbon center in the starting material than to install it there by de novo means later in the synthesis (although this could presumably have been accomplished via a C_9−C_{11} olefin). Given these considerations, we settled on 11-keto-androstene-3,17-dione (**6**) as our starting material. It was appreciated that the distance to be traversed in getting from **6** → **1** is quite substantial. We felt that this starting material offered larger advantages of flexibility which more than compensated for passing up more elaborated structures whose added complexity could have served, in our judgment, to narrow our available options.

Construction of 6-keto-7β-OH Ketol. A critical final stage of our proposed synthesis would require a potentially nontrivial installation of the C_6-keto-C_7−β-OH arrangement. Early in the project, we sought to establish suitable possible reaction parameters for this type of objective, using as a model the structurally simplified synthetic intermediate, **11**. The latter was prepared from the commercially available adrenosterone (**6**) in six steps, as shown (Scheme 1). Thus, **6** was subjected to a three-step sequence of acetylation, selective ketalization of the C_{17} ketone, and $NaBH_4$ reduction to afford the 3β,11β-diol **7**.[4] Upon TBS protection of the free alcohols, intermediate **8** was in hand. Treatment of **8** with CrO_3-dimethylpyrazole led to allylic oxidation at C_7, furnishing ketone **9** in moderate yield.[5] Selective 1,2-reduction of enone **9** under Luche conditions[6] gave the 7β-OH steroid **10** as the only detectable epimer in high yield.[7] Finally, treatment of **10** with BH_3-THF and subsequent oxidative workup with H_2O_2 led to the formation of key intermediate **11**.

SCHEME 2. Synthesis of 6α-OH-7β-OTBS Steroid 14[a]



[a] Conditions: (a) TBSCl, Im., DMAP, DMF, 86%; (b) BH_3-THF, THF, phosphate buffer; NaOH, H_2O_2, 13%.

Our first attempts to install the C_6-keto-C_7−β-OH ketol focused on the strategy of selective MPM ether protection of the 7β-OH of **11**. Unfortunately, however, all of our efforts to form *p*-anisyl acetal **12** from 6α,7β-OH steroid **11** were unsuccessful. This failure may be attributed to the significant steric hindrance of the trans disposed 6,7-diol as well as the potential susceptibility of the *p*-anisyl acetal to acidic conditions.

We next investigated a strategy wherein the 7β-OH group would be protected prior to introduction of the C_6-hydoxyl group. Thus, intermediate **10** was protected as the TBS ether to afford **13** (Scheme 2). Unfortunately, hydroboration of **13** gave rise to intermediate **14** in only 13% yield. The low yield may be ascribed to possible migration of the TBS functionality from the 7β-OH to the 6α-OH group. Indeed, a similar silyl migration pattern with a 6,7-dihydroxysteroid has been reported by Jung et al. in their synthesis of xestobergsterols.[8]

We next considered the possibility of forming the ketol through an intermediate epoxide. Thus, **10** was treated

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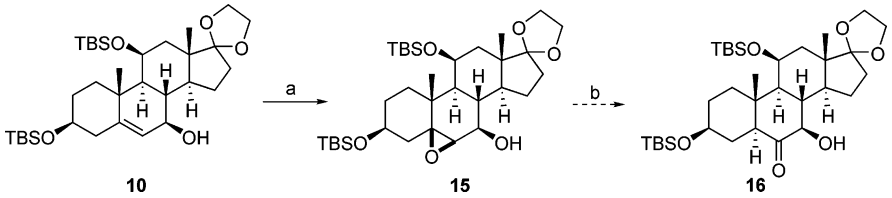
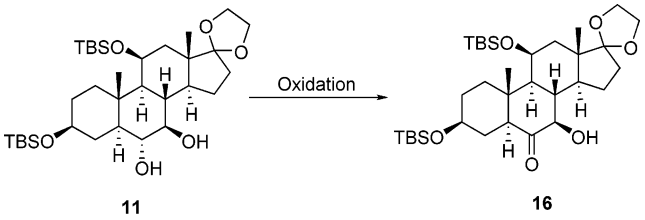
SCHEME 3. Attempted Epoxide Rearrangement to 6,7-ketol Steroid 16[a]



[a] Conditions: (a) mCPBA, CH_2Cl_2, 0 °C to room temperature; (b) $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt.

SCHEME 4. Synthesis of 6-keto-7β-OH Steroid 16



Conditions	Result
PCC, CH_2Cl_2, rt	No reaction
Ag_2CO_3, celite, DMF, 80 °C	Messy
Dess-Martin periodinane, CH_2Cl_2, rt	Single product

with mCPBA to afford **15**, wherein the allylic 7β-hydroxyl group directed exclusive β-face epoidation of the molecule (Scheme 3). We now hoped to effect a rearrangement to the ketol **16**; however, treatment of **15** with $BF_3{\cdot}Et_2O$ led only to decomposition.

Finally, we decided to attempt the direct selective oxidation of the 6,7-diol, **11**. We were encouraged by a protocol developed by Krafft in their studies toward the synthesis of xestobergsterol A[9] which allowed exclusive benzylation of 6α-OH in the presence of 7β-OH. Our initial attempt, using PCC as the oxidizing agent, was unsuccessful, leading only to recovery of the starting material (Scheme 4). Treatment of **11** with silver carbonate and Celite yielded a mixture of products. Gratifyingly, however, the reaction of **11** with Dess−Martin periodinane afforded a single ketol product, which we believed to be the desired **16**. Our provisional assignment was based on a working hypothesis that an appropriate oxidizing reagent would approach the hydroxyl group only from the sterically less hindered α face, resulting in the selective oxidation of the 6α-OH group.

To confirm the structure of **16**, we prepared the 6α-OH-7-keto steroid (**19**). Thus, selective benzoylation of **11** under Krafft's conditions afforded the monobenzoylated intermediate **17**, as expected (Scheme 5). Oxidation of the 7β-OH with Dess−Martin periodinane afforded **18**, which, upon deprotection, gave rise to the 6α-OH-7-keto steroid **19**. Comparison of the two ketol steroids, **19** and **16**, by ^{1}H NMR revealed two different compounds, thus confirming that the product formed in the direct oxidation was indeed the desired keto steroid **16**. We could now be confident that we had established suitable conditions for the late-stage installation of the 6-keto-7β-OH functionality directly from the 6α,7β-diol.

Synthesis of Δ17(20)-16-one Steroid 25. Having established, in principle, a means for late-stage oxidation of the 6,7-trans diol, we returned to the synthesis of the natural product. From intermediate **11**, the next task would be that of introducing the C_{17} side chain with the correct C_{17} and C_{20} stereochemistry. We sought to accomplish this subgoal through cuprate addition to a structure of the type **25**. Thus, treatment of **11** with acetic acid led to removal of the C_{17} ketal with concomitant cleavage of the C_3 TBS silyl ether to afford **20** (Scheme 6). Fortunately, the C_6 and C_7 hydroxyl groups could be engaged as an acetonide containing the isopropylidene protecting group.

Wittig olefination of **21** proceeded with excellent selectivity to afford the (*Z*)-17(20) ethylidene **22** in 66% yield.[10] The C_3 hydroxy group was then reprotected as the TBS ether, affording **23** in quantitative yield. The latter compound was subjected to SeO_2 catalyzed allylic oxidation to give rise to **24**, which, upon MnO_2 oxidation, afforded the geometrically defined enone intermediate **25**. (17*E*) and (17*Z*)-17(20)-en-16-one steroids are configurationally unstable in solution.[11] However, immediate chromatographic purification of the crude compound on silica gel column gave the desired (17*E*) steroid (**25**) as the major product with no observed isomerization. Thus purified, **25** is configurationally stable and can be stored in the solid state.

Synthesis of the Side Chain and Conjugate Addition. Vinyl iodide **29**, which would serve as the coupling partner in the side chain installation, was prepared in three steps from commercially available ergosterol (**26**). Thus, acetylation of **26**, followed by ozonolysis, provided the (*R*)-2,3-dimethylbutanal **28** in moderate yield (Scheme 7).[12] Although this protocol for the preparation of aldehyde **28** is hardly "atom economical,"[13] the synthesis requires only two steps and uses inexpensive starting materials (ergosterol: $126/25 g). Aldehyde **28** was then converted to vinyl iodide **29** according to the Takai olefination protocol.[14] Compared to the same reaction of 3-methylbutanal,[15] the *E*/*Z* ratio of the vinyl iodide product was significantly improved from 4:1 to 93:7. The boiling point of vinyl iodide **29** is rather low (~60 °C), which makes it impractical to evaporate the solvent completely. Column chromatography on **29** was performed with the low boiling solvent, pentane, as the eluant, and the resultant product solution was concentrated and could be stored as a pentane

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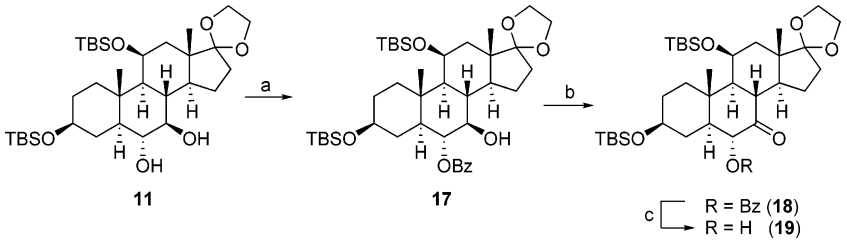
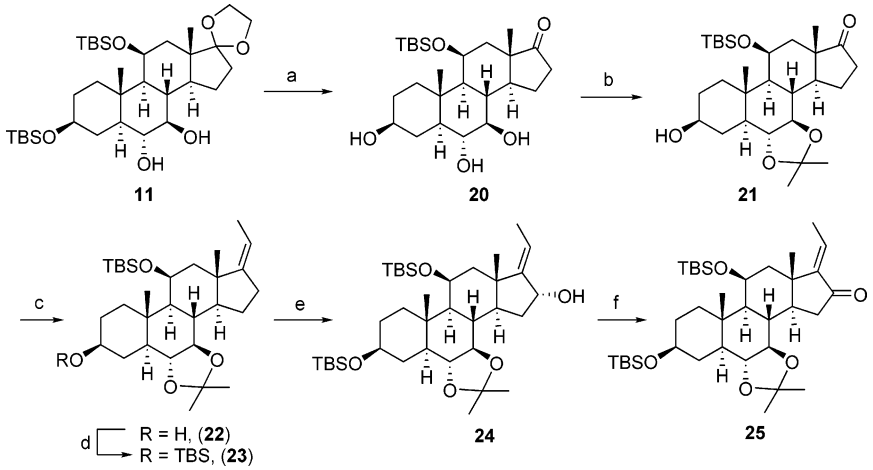
SCHEME 5. Synthesis of 6α-OH-7-keto Steroid 19[a]



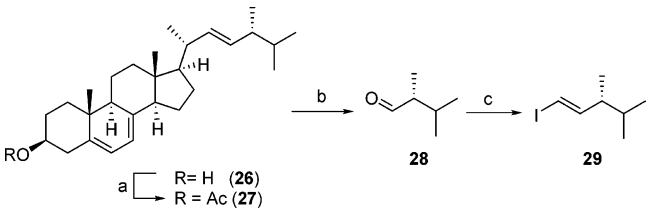
[a] Conditions: (a) BzCl, Et_3N, DMAP, CH_2Cl_2, 0 °C to room temperature, 4.5 h; (b) Dess−Martin periodinane, CH_2Cl_2, rt, 1 h; (c) 1% KOH, MeOH, rt, 2.5 h.

SCHEME 6. Synthesis of Δ17(20)-16-one Steroid 25[a]



[a] Conditions: (a) AcOH-H_2O (4:1), rt, 2.5 h, 82%; (b) $Me_2C(OMe)_2$, CSA, DMF, rt, 6h, 93%; (c) $EtPPh_3Br$, KO^{t}Bu, THF, reflux, 66%; (d) TBSOTf, 2,6-lutidine, CH_2Cl_2, rt, quant.; (e) SeO_2, TBHP, CH_2Cl_2, 0 °C to room temperature, 6 h; (f) MnO_2, CH_2Cl_2, reflux, 3.5 h, 43% (two steps).

SCHEME 7. Synthesis of Vinyl Iodide 29[a]



[a] Conditions: (a) Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt, 12 h; (b) O_3, pyridine, CH_2Cl_2, −78 °C; then Me_2S, rt. 38% (two steps); (c) $CrCl_2$, CHI_3, THF, 0 °C to room temperature, 4 h, 45% (*E*:*Z* = 93:7).

solution. However, best yields in subsequent coupling reactions were obtained with freshly prepared material.

With enone **25** and vinyl iodide **29** in hand, the installation of the C_{17} side chain via cuprate conjugate addition was studied. Treatment of vinyl iodide **29** with *t*-BuLi afforded the vinyllithium agent which, upon treatment with CuCN, yielded high-order vinyl cuprate species, **30**. Stereoselective addition of the vinyl cuprate from the *Si* face of the Δ17(20)-16-one, followed by kinetic protonation of the resultant enolate from the same face, furnished steroid **31** with the desired stereochemistry at both C_{17} and C_{20} (Scheme 8).

First Attempted Synthesis of NGA0187. With the C_{17} side chain stereoselectively installed, several functional group modifications would be required for the completion of the synthesis of NGA0187. First, the C_{16} ketone of **31** was stereoselectively reduced with $LiAlH_4$ to afford the 16*β*-OH steroid **32**, which was subsequently acylated to produce **33** (Scheme 9).[16] Standard aqueous acidic conditions (TsOH, PPTS, $Pd(CH_3CN)_2Cl_2$, etc.) for acetonide removal resulted in concomitant cleavage of the C_3 TBS ether. However, a slightly modified Williams' procedure[17] of ketal exchange with 1,2-ethanedithiol in the presence of catalytic amounts of CSA in anhydrous chloroform selectively removed the 6,7-acetonide to give steroid **34** containing free hydroxy groups at C_6 and C_7. The previously developed protocol for selective oxidation of the 6α,7*β*-diol to the 6-keto-7*β*-OH steroid proceeded smoothly to afford **35** as a single detectable product. Unfortunately, however, the removal of the TBS protecting groups proved troublesome. Although the C_3 TBS ether could be easily cleaved, the C_{11} TBS ether remained intact under all conditions attempted. The difficulty in removing the C_{11} TBS ether may be explained by the significant steric hindrance caused by the axial methyl groups projecting from C_{10} and C_{13}. To complete the synthesis of NGA0187, it would be necessary to reconsider our protecting group selections.

In light of these findings, we decided to replace the C_{11} protecting group with a more labile TES function. We first attempted to change the protecting groups on the relatively late stage intermediate **22**; however, the C_{11}

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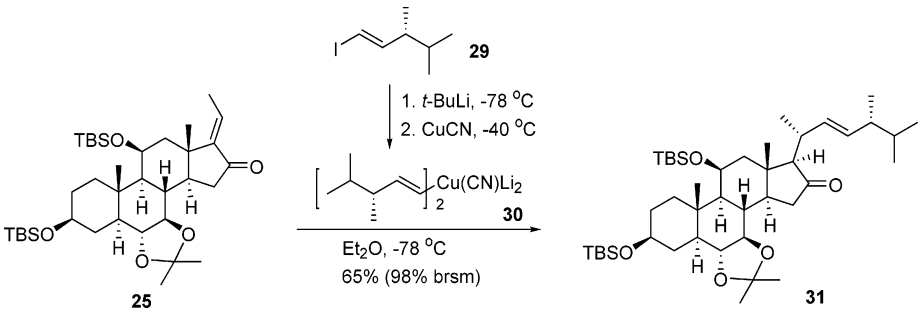
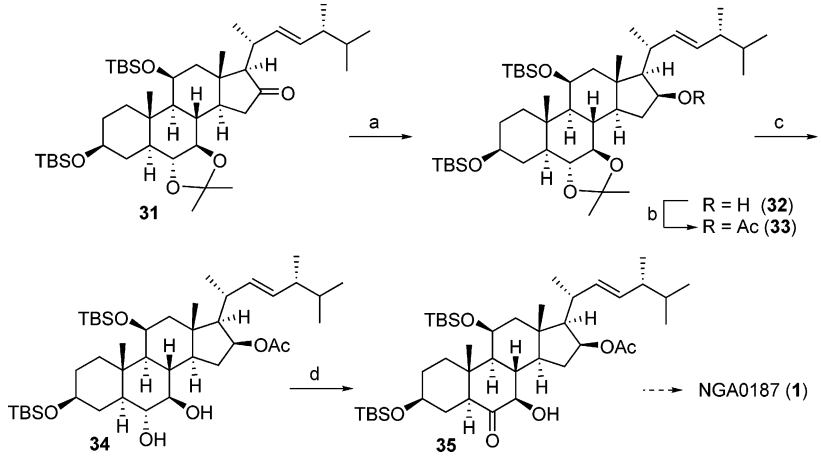
SCHEME 8. Conjugate Addition

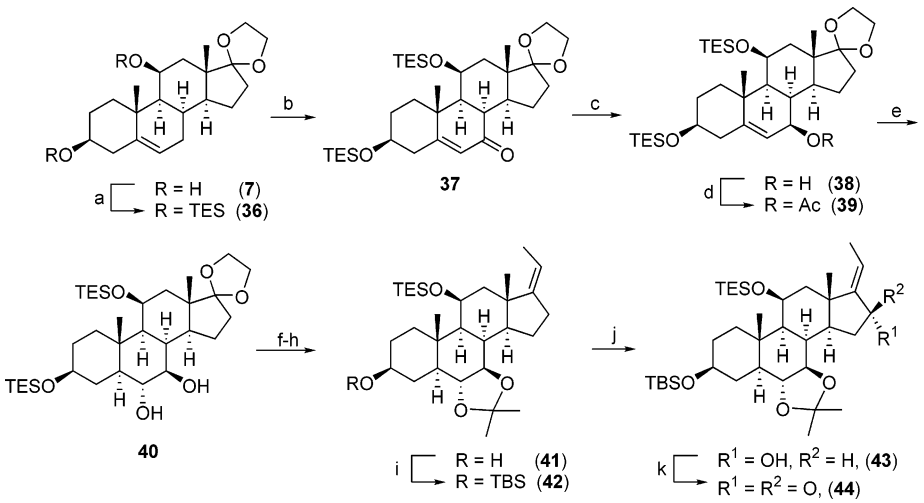


SCHEME 9. First Attempted Synthesis of NGA0187[a]



[a] Conditions: (a) $LiAlH_4$, THF, −78 °C; (b) Ac_2O, DMAP, pyridine, 0 °C to room temperature; (c) $(CH_2SH)_2$, CSA, $CHCl_3$, reflux; (d) Dess−Martin periodinane, CH_2Cl_2, rt.

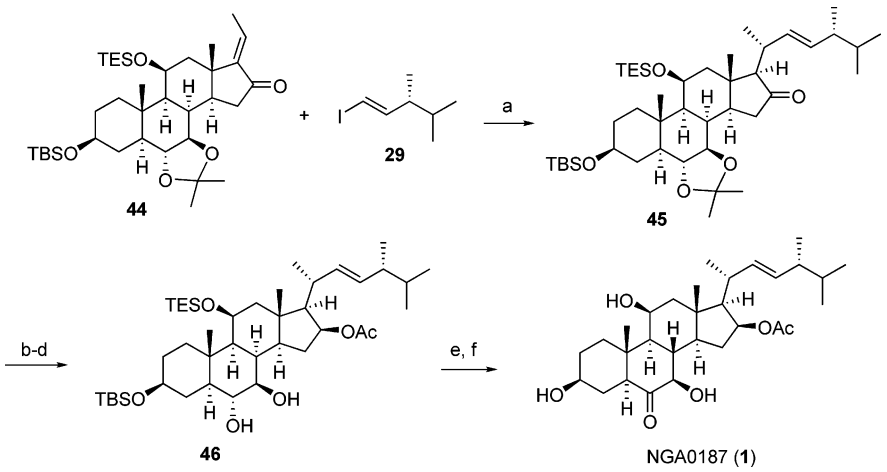
SCHEME 10. Synthesis of C-11-TES Steroid 44[a]



[a] Conditions: (a) TESOTf, pyridine, CH_2Cl_2, 0 °C, 1 h, 87%; (b) CrO_3, 3,5-dimethylpyrazole, CH_2Cl_2, −25 °C, 4 h, 51%; (c) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, THF-MeOH, −70 to −30 °C, 1 h, 96%; (d) Ac_2O, DMAP, pyridine, CH_2Cl_2, rt, 4 h, 98%; (e) BH_3-THF, THF, 0 °C to room temperature, 12 h; NaOH, H_2O_2, 2 h, rt, 76%; (f) AcOH-H_2O, rt, 2.5 h; (g) 2, 2-dimethoxypropane, CSA, DMF, rt, 8 h; (h) $EtPPh_3Br$, KO^tBu, THF, reflux, 3 h, 67% (three steps); (i) TBSOTf, 2,6-lutidine, CH_2Cl_2, rt, 1 h, 98%; (j) SeO_2, *t*-BuOOH, CH_2Cl_2, 0 °C, 5 h, 72%; (k) Dess−Martin periodinane, CH_2Cl_2, rt, 1 h, 98%.

ether was resistant to cleavage under several conditions attempted. We were then forced to return to the early stage diol intermediate **7**. Thus, compound **7** was converted to the di-TES ether **36**. The latter was subjected to allylic oxidation to afford **37**, which was selectively reduced to give rise to **38**. Subsequent hydroboration of **38** with oxidative workup provided diol **40** in moderate yield (47%). The observed yield is lower than that obtained with the TBS ether substrate (81%) because of formation of the 6β,7β-diol byproduct. Alternatively, alcohol **38** was first acetylated to afford **39**. Upon exposure to the hydroboration-oxidation conditions, in-

SCHEME 11. Completion of the Synthesis of NGA0187 (1)[a]



[a] Conditions: (a) **29**, *t*-BuLi, −78 °C, Et_2O, 0.5 h, then CuCN, −40 °C, 0.5 h; **44**, −70 °C, 2 h, 61% (93% brsm); (b) $LiAlH_4$, THF, −78 °C, 1 h; (c) Ac_2O, DMAP, Py., 0 °C, 4 h; (d) $(CH_2SH)_2$, CSA, $CHCl_3$, reflux, 1 h, 61% (three steps); (e) Dess−Martin periodinane, CH_2Cl_2, rt, 3 h; (f) HF-CH_3CN-H_2O, rt, 5 h, 61% (two steps).

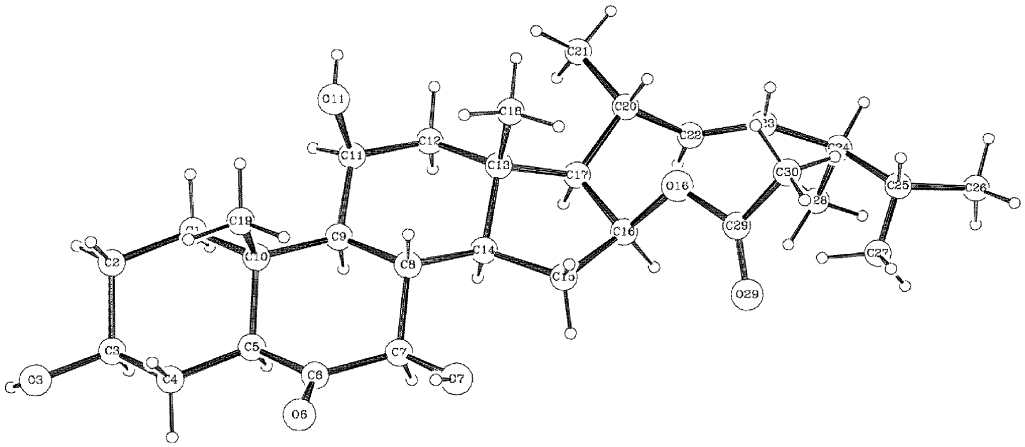


FIGURE 2. ORTEP drawing of NGA0187.

termediate **39** was converted to diol **40** in 76% yield. Treatment of **40** with acetic acid led to removal of the C_{17} ketal and cleavage of the C_3 TES functionality. The resultant triol was converted to intermediate **41** through treatment with 2,2-dimethoxypropane in the presence of catalytic amounts of CSA, followed by selective Wittig olefination. The C_3 hydroxy group was then converted to the TBS ether, **42**. SeO_2 catalyzed oxidation furnished allylic alcohol **43**, which was subsequently oxidized to **44** under Dess−Martin oxidation conditions.

Completion of the Synthesis of NGA0187. The TES-protected steroid **44** was further advanced, according to the chemistry described above for the conversion from **31** to **35**. Thus, conjugate addition of the vinyl cuprate generated from **29** to steroid **44** afforded **45** with good yield (Scheme 11). Reduction of the C_{16} ketone, acetylation of the resultant 16β-OH, and removal of acetonide through ketal exchange with thiol afforded 6α,7β-diol steroid **46** in 61% yield over three steps. Subjection of **46** to Dess−Martin periodinane in CH_2Cl_2 at room temperature led to the selective oxidation of the 6α-OH to the ketone. Finally, treatment of the crude ketol steroid with 48% HF in CH_3CN (1:9) led to cleavage of both TBS at C_3 and TES at C_{11} to give the natural product, NGA0187 (**1**). The overall yield for 20 steps from adrenosterone (**6**) is 3%. The structure of synthesized NGA0187 was analyzed by ^1H, ^{13}C, DEPT, COSY, and HMQC NMR techniques ($CDCl_3$ was used as solvent instead of the originally used d_6-DMSO in isolation paper). Moreover, to avoid any chance for error, the structure was unambiguously confirmed by X-ray diffraction on the fully synthetic material (shown in Figure 2).

Biological Evaluation of Synthesized NGA0187 and Analogues. We were now prepared to verify the neurotrophic activity previously claimed for NGA0187. The ability of synthetic NGA0187 to stimulate NGF-mediated neurite outgrowth was evaluated by treating rat pheochromocytoma cells (PC12) with NGF (50 ng/mL) and NGA0187 (30 μM) for 96 h. The cells were then compared to a similarly prepared control, which lacked only the NGA0187. As shown in Figure 3, although no neurite growth was observed in the control sample (picture A), the sample treated with 30 μM NGA0187 demonstrated considerable sprouting (picture B).

Having corroborated the earlier reported neurotrophic activity, we next attempted to establish a very preliminary SAR map for NGA0187. As the ultimate objective

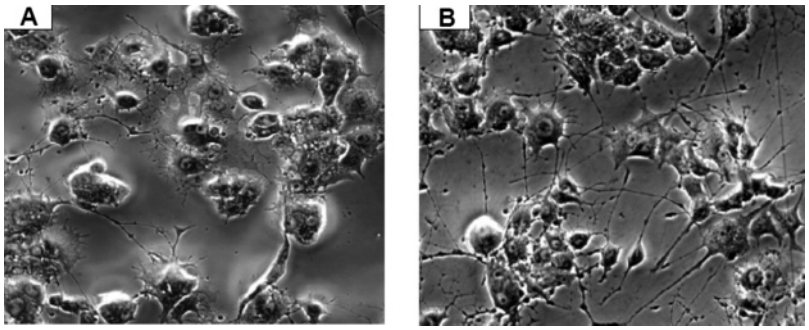
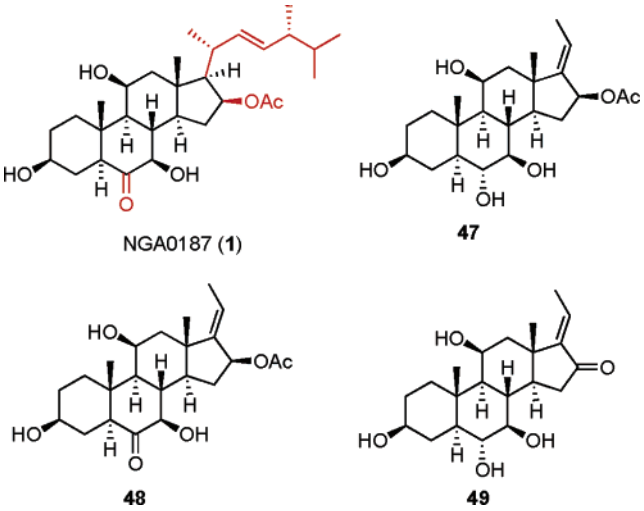


FIGURE 3. Images of neurons after treatment with (A) DMSO + NGF, (B) NGA0187 in DMSO (30 μM) + NGF.

of drug discovery is the development of biologically active, structurally simplified analogues, we decided to examine the effects of removing the C_{17} side chain. In addition, we hoped to evaluate in a preliminary way the impact of manipulating the oxidation states of the C_6 and C_{16} hydroxyl components. Thus, three structurally simplified analogues, **47**, **48**, and **49**, were prepared and evaluated at 30 μM under conditions similar to those employed in the biological evaluation of NGA0187 itself.



Interestingly, none of these analogues promoted neurite outgrowth to any appreciable extent (Figure 4). The lack of activity of compound **48** is especially informative, as this analogue differs from NGA0187 only in the C_{17} side chain. These results suggest that the C_{17} side chain possibly plays a critical role in target recognition.

Compound **1** (NGA0187) significantly promoted (269%) neurite outgrowth relative to the DMSO control ($^*P < 0.001$). Compound **49** promoted differentiation about 123% comparing to control, but it is not significant ($P > 0.5$). Compounds **47** and **48** do not have any effect.

In retrospect, the decision to begin with compound **6** eventually worked out very nicely, allowing us to exploit the reasonably well understood steroidal framework to selectively process functionality at different points with high stereoselection. We are now confident that synthesis, while nontrivial, will provide for us access to substrates with which to map an informative SAR profile.

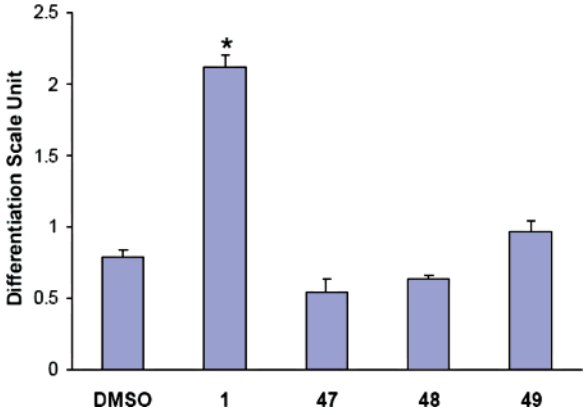


FIGURE 4. Evaluation of neurite growth. Value represents mean ± SE for 10 views from 5 wells.

Certainly, the prospect of using a steroid framework as a matrix for attaching pharmacophores to enhance neurotrophic activities is an attractive one. The loss of activity with some of the derivatives described above lacking the C_{17}-side chain underscores the need to pinpoint the structural specificity required for activity. This will be necessary to foster additional potency before nominating one of this family of compounds for serious clinical development.

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Supporting Information Available: Experimental procedures and characterization data for compounds **1**, **8**−**11**, **16**−**19**, **22**, **23**, **25**, **29**, **31**, **34**, and **36**−**46** and CIF file for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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