Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 1, pp 125–133, 1997

PII: S0968-0896(96)00202-7
0968-0896/97 $17.00+0.00

Ras Oncoprotein Inhibitors: The Discovery of Potent, Ras Nucleotide Exchange Inhibitors and the Structural Determination of a Drug–Protein Complex

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Abstract—The nucleotide exchange process is one of the key activation steps regulating the ras protein. This report describes the development of potent, non-nucleotide, small organic inhibitors of the ras nucleotide exchange process. These inhibitors bind to the ras protein in a previously unidentified binding pocket, without displacing bound nucleotide. This report also describes the development and use of mass spectrometry, NMR spectroscopy and molecular modeling techniques to elucidate the structure of a drug–protein complex, and aid in designing new ras inhibitor targets. Copyright © 1997 Elsevier Science Ltd

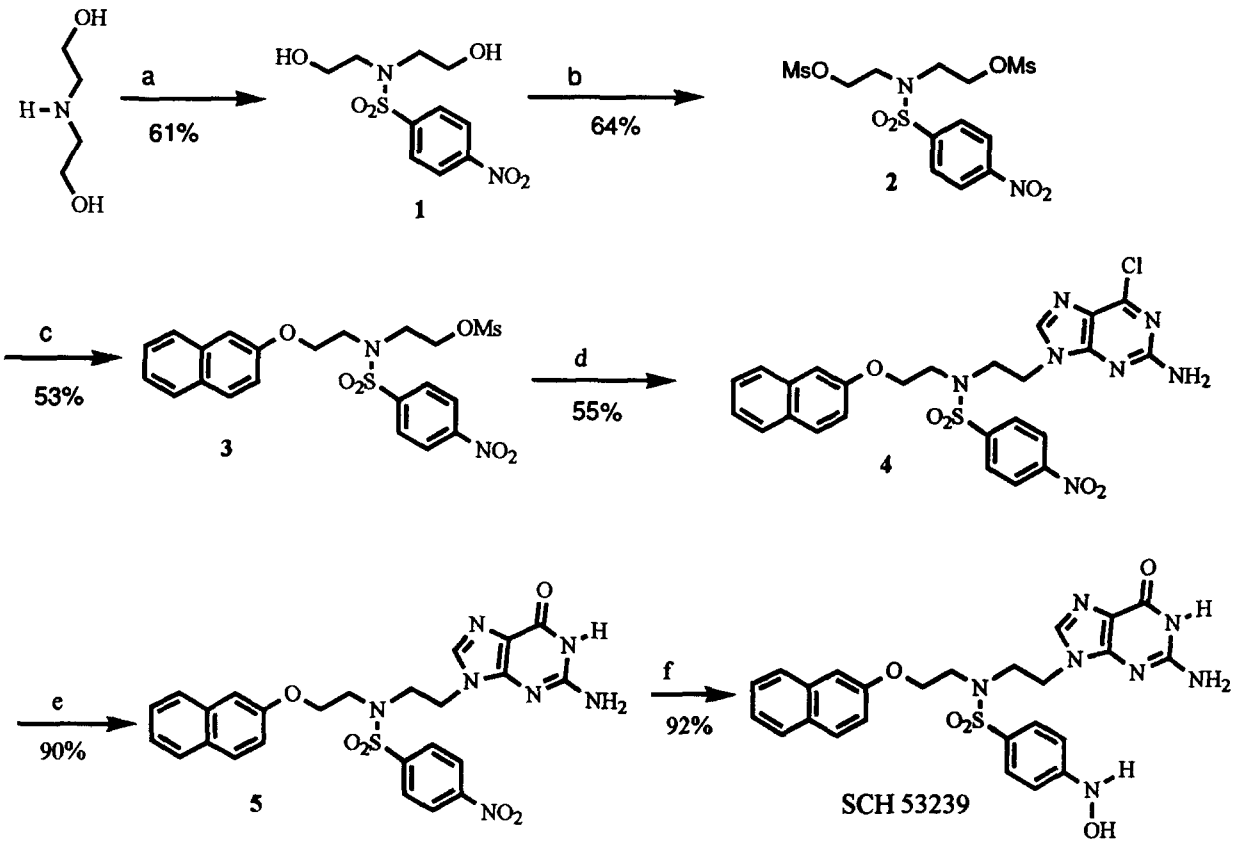
Nearly 20% of all human tumors are linked to a mutation of the ras oncogene where the greatest associations between ras and human tumors have been found in lung (40%), pancreatic (90%) and colon (50%) carcinomas.[1] The ras protein mediates cell proliferation by linking an extracellular growth factor with the nucleus via signal transduction involving an activation–deactivation cycle. Activation of non-mitogenic ras-GDP to mitogenic ras-GTP is stimulated by membrane-associated tyrosine kinases and nucleotide exchange factors.[2] Inhibition of GDP–GTP nucleotide exchange of mutated, tumorigenic ras could prevent continued cell growth and hence, could represent a novel treatment for cancer.

Our overall objective was to develop compounds that would bind to the ras protein, at a site other than the nucleotide binding site, and deactivate ras by preventing the exchange of GTP for GDP.[3] In this report we disclose our efforts toward designing and synthesizing nucleotide exchange inhibitors of the ras protein based on the x-ray crystallographic structures of ras-GDP[4c] and ras-GTP. Numerous attempts to co-crystallize our lead structures with ras-GDP were unsuccessful. However, as will be indicated from our NMR and mass spectral data, SCH-54292 does indeed bind to ras-GDP and the drug–protein complex in solution has been modeled using its associated NOEs in conjunction with the crystallographically determined ras-GDP structure.[4c,d]

Synthesis

The synthesis of SCH-53239 is outlined in *Scheme 1*. Bis(hydroxyethyl)sulfonamide (**1**) was prepared by condensation of *para*-nitrobenzenesulfonyl chloride and bishydroxyethylamine. Per-mesylation with methanesulfonyl chloride and triethylamine in dichloromethane afforded sulfonamide **2** in 64% isolated yield. Selective displacements of the mesylate functions in **2** were effected by treatment with 1 equivalent of 2-naphthol and sodium hydroxide in aqueous acetone followed by 1 equivalent of 2-amino-6-chloropurine and potassium carbonate in hot *N*,*N*-dimethylformamide (DMF). Aqueous acid hydrolysis of the resulting chloropurine (**4**) afforded guanine (**5**) (90%). Catalytic hydrogenation of the nitro function in (**5**) provided *N*-hydroxysulfanilamide SCH-53239 in 92% yield.

N-Hydroxysulfanilamides SCH-53870 and SCH-54292 were prepared as illustrated in *Scheme 2*. Accordingly, sulfonylation of 2-(2-naphthyloxy)-ethylamine[5] with *para*-nitrobenzenesulfonyl chloride in triethylamine and dichloromethane provided sulfonamide **7**. Palladium-catalysed hydrogen transfer from hydrazine[6] smoothly effected the conversion of the nitro substituent in **7** to the hydroxylamine function in SCH-53870 (94%). Alternatively, deprotonation of sulfonamide **7** with sodium hydride in DMF and alkylation of the resulting anion with acetobromo-α-D-glucose provided tetra-acetoxy **8**, which upon treatment with aqueous potas-

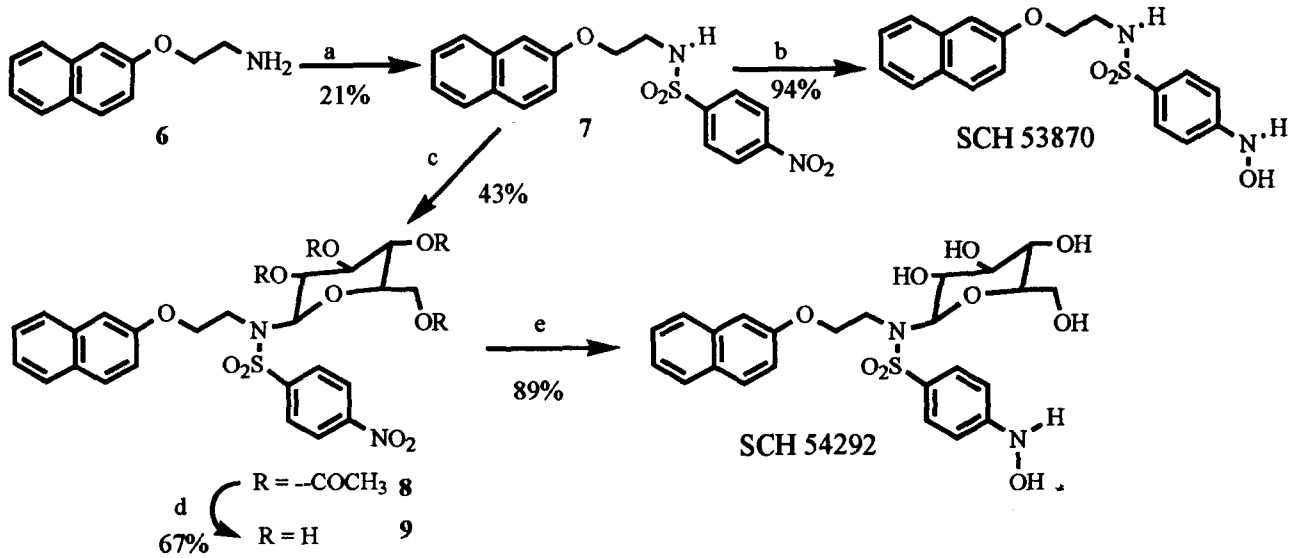


Scheme 1. Reagents and conditions: (a) 4-NO_2PhSO_2Cl, NEt_3, CH_2Cl_2; (b) CH_3SO_2Cl, NEt_3, CH_2Cl_2; (c) 2-naphthol, NaOHJ, H_2O, acetone; (d) 2-amino-6-chloropurine, K_2CO_3, DMF; (e) 12 N HCl, acetic acid; (f) H_2, 10% Pd/C, DMF.

sium carbonate in methanol afforded sulfonamidoglycoside **9** in 67% yield. Palladium-catalysed transfer hydrogenation of **9** yielded *N*-hydroxysulfonylamide SCH-54292 (89%).

Results and Discussion

The ras nucleotide exchange assay used in our studies measures the extent of exchange of P^{32}-GDP by unlab-



Scheme 2. Reagents and conditions: (a) 4-NO_2PhSO_2Cl, NEt_3, CH_2Cl_2; (b) NH_2NH_2, 5% Pd/C, EtOH, THF; (c) NaH, DMF, Acetobromo-α-D-glucose; (d) K_2CO_3, MeOH, H_2O; (e) NH_2NH_2, 5% Pd/C, EtOH, THF, DMF.

eled GDP in the tumorigenic valine-12 mutant form of ras in the presence of an inhibitor.[7] Under these conditions GDP had an $IC_{50}=0.5\mu M$. SCH-53239, originally designed to bind competitively with GDP in the nucleotide binding site of the ras protein, also had an $IC_{50}=0.5\ \mu M$. Subsequent structure activity relationship (SAR) studies led to the development of SCH-53870 ($IC_{50}=\ 0.5\mu M$) and a derivative with greater water solubility, SCH-54292 ($IC_{50}=0.7\mu M$). The water solubility of SCH-54292 was 0.35 mM while SCH-53870 was only sparingly soluble ($<0.1\ \mu M$). Scatchard analyses showed that the binding of SCH-53239 and of SCH-54292 to ras was not competitive with the binding of GDP. To explore further the binding interactions between ras-GDP and SCH-53239, SCH-53870 and SCH-54292, we attempted to isolate co-crystals suitable for X-ray crystallographic analysis of each complex. We were unsuccessful and subsequently utilized mass spectral, NMR and molecular modeling applications in order to elucidate the nature of the interactions between the ras protein and these inhibitors.

Electrospray mass spectroscopy has been reported by our laboratories to be a powerful analytical tool for identifying ras protein complexes.[8,9] Electrospray mass spectral analysis of a solution containing ras-GDP (19295 Da) and SCH-54292 revealed a peak (19816 Da) corresponding to a ternary, non-covalent complex of ras-GDP–SCH-54292 without any evidence for a signal corresponding to a dimeric species (19373 Da) representing ras–SCH-54292 .

Additional information about the three-dimensional structures of the ras protein complexes and the binding site involved in the complexation of SCH-54292 was obtained from a technique which indicated the extent of succinoylation of amine functions in ras-GDP, ras-GDP–SCH-54292 and denatured ras following treatment with succinic anhydride.[10,11] Each succinoylated protein structure was denatured and proteolyzed with endoproteinase Lys-C,[12] and the resulting collection of succinoylated and non-succinoylated peptide fragments was analysed by LC-MS and compared with the known sequence of ras[13] to reveal the specific locations of the derivatized lysines. Only lysine amines and amino terminus functions exposed to succinic anhydride were derivatized while unmodified lysines not embedded within the folded protein structure of ras-GDP[3c] were, *vide infra*, involved in the binding of GDP and of SCH-54292.[11]

Derivatization of ras-GDP–SCH-54292 occurred mainly at Lys-42, Lys-104 and at the amino terminus while Lys-16, Lys-117 and Lys-147 (residues involved in GDP binding) were fairly well-protected from succinoylation. Lys-101, located on the underside of the cleft in the Switch-2 region, was protected from derivatization in the ras-GDP–SCH-54292 complex while this residue in ras-GDP was modified extensively by succinic anhydride. Lys-5 and Lys-88, located within the naturally folded protein structures of ras-GDP (as determined by x-ray crystallography)[4c] and ras-GDP–SCH-54292 were not modified. All 9 amine functions (8 lysine amines and 1 amino terminus)[13] of denatured ras were succinoylated.

Complexation of SCH-54292 with ras-GDP was also observed by NMR spectroscopy where negative NOEs, characteristic of large molecules and complexes, have been recorded for the intra- and intermolecular interactions of SCH-54292 with ras-GDP.[14a] Additionally, the resonances corresponding to SCH-54292 bound to ras-GDP were broad compared to those of free inhibitor, presumably due to the shorter relaxation time of the inhibitor in the resulting complex (see *Fig. 1*).[14b]

Analysis of the intramolecular NOEs for SCH-54292 bound to ras-GDP afforded the interatomic distances shown in Table 1. A Monte Carlo analysis of this data, allowing for tolerances of 0.4 Å,[15] provided an energetically accessible bioactive conformation of SCH-54292 bound to ras-GDP (*Fig. 2*).

Three intermolecular NOEs are evident in the 2-D NOESY spectra of the complex between SCH-54292 and ras-GDP and are attributed to protons Hc1 and Hc3 of the naphthyl moiety of SCH-54292 (see *Fig. 3*). The protons of the amino acid residues of ras-GDP with which these protons interact resonate at 2.00–2.10, 1.56–1.66, and −0.014–0.86 ppm. While many amino acid residues of ras-GDP have associated protons which resonate at these chemical shifts,[16,17] two

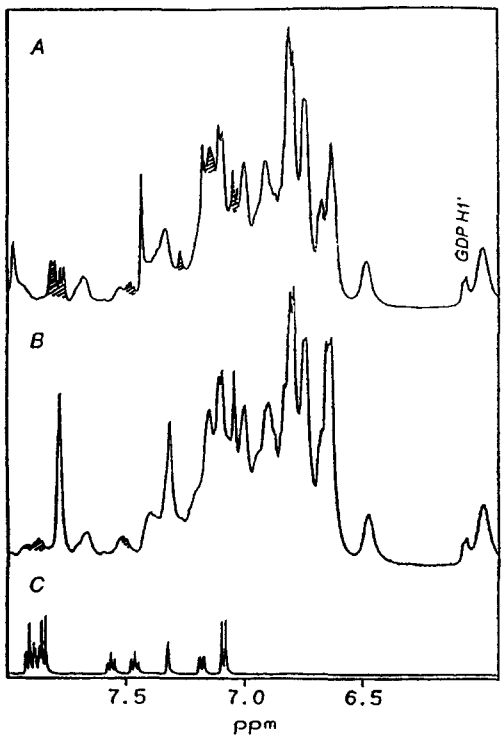


Figure 1. Binding of SCH-54292 to the ras protein. The low field regions of the proton NMR spectra of the ras protein in complex with SCH-54292 prepared by mixing at 0.3 mM [Mg^{2+}] and adjusting [Mg^{2+}] to >1mM (A), by mixing at 1 mM [Mg^{2+}] (B), and the spectrum of free SCH-54292 (C). The shaded areas in (A) and (B) indicate the bound SCH-54292.

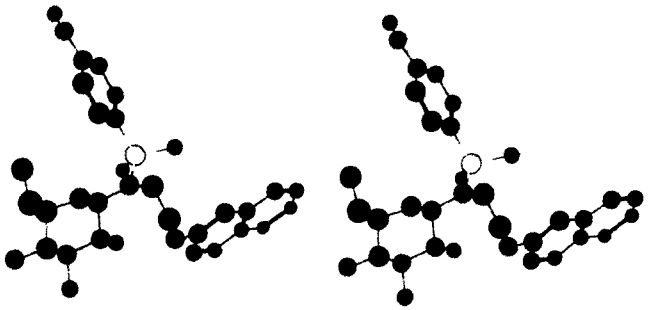


Figure 2. The conformation of SCH-54292 bound to ras-GDP (C, gray; N, blue; O, red; S, yellow).

ras protons simultaneously interacting with Hc1 and Hc3 of SCH-54292 must reside *within* a specific distance as defined in Figure 3. Examination of all possible binding orientations of the calculated, bound conformation of SCH-54292 with ras-GDP,[4c,d] taking into account the observed intermolecular NOE interactions, produced two possible models of the ras-GDP–SCH-54292 complex. Energy minimizations[18] of one of these models resulted in a conformation of SCH-54292 which no longer satisfied the NOE constraints. The other model, where Hc1, Hc1, and Hc3 of SCH-54292 are located near amino acid protons Ile100-HCg 11, Ile100-HCg 12 and Arg68-HCb of ras-GDP, respectively, is consistent with the geometrical and spectral criteria.

This model, reproduced in Figure 4, shows SCH-54292 bound to ras-GDP in a major cleft comprising the Switch-2 region (residues 60–76) which is flexible and changes its conformation depending on whether GDP or GTP is associated with ras.[4] The hydroxylamine moiety of SCH-54292 is situated near the Mg^{2+} cation and may potentially serve as a chelator thereby enhancing its binding affinity. The glucose substituent, while enhancing the solubility of the inhibitor, is believed not to contribute to binding, a proposal supported by the nearly equivalent potency of the des-glucosyl derivative SCH-53870 in the nucleotide exchange enzyme assay. The mass spectral data which showed Lys-101 to be protected from succinoylation is consistent with this model. The shielding of Lys-147 from succinoylation may be due to a shift of GDP in the ras-GDP–SCH-54292 complex.

The ras-related cellular effects of SCH-54292, SCH-53239 and SCH-53870 have also been studied.

Table 1. NOE-derived interatomic distances of SCH-54292 bound to ras-GDP. Atom assignments are found in Figure 3

Hz-H1	3.6 Å
Hz-H2	3.5–5.0 Å
Hz-H5	3.5–5.0 Å
Hz-H4	3.5–5.0 Å
Hz-H6	3.5–5.0 Å
Hz-Hf	≥4 Å
Hf-Hja or Hjb	2.5 Å
Hf-Hjc or Hjd	4.0 Å
Hc1 or Hc3-Hja or Hjb	4.0 Å

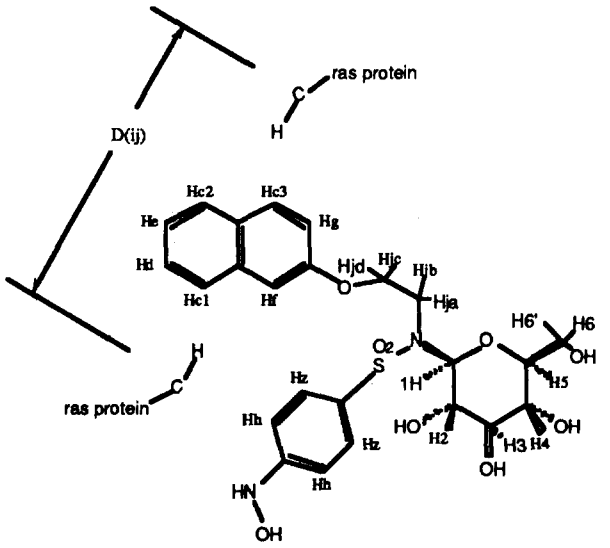


Figure 3. Schematic representation of two of the three ras protons simultaneously exhibiting NOEs with Hc1 and Hc3 of SCH-54292. The distance between any pair of these ras carbon atoms is defined by $D(ij) \leq \Sigma D_{\text{Hc1-Hc3}} + 2D_{\text{C-H}} + D_{\text{NOE}(i)} + D_{NOE(j)}$ where $D_{\text{Hc1-Hc3}}$ is the distance between Hc1 and Hc3 of SCH-54292 (=5.52 Å), $D_{\text{C-H}}$ is a ras carbon–hydrogen bond length (=1.1 Å), and $D_{\text{NOE}(i)}$ and $D_{\text{NOE}(j)}$ are the calculated distances from the integrated interatomic NOEs observed between protons of ras-GDP and Hc1 and Hc3 of SCH-54292. Using this equation, the maximal distances for the three pairs of carbon atoms must be less than 18.06, 18.56 and 19.16 Å.

Ras nucleotide exchange has been linked to NGF induced neurite outgrowth of PC-12 cells.[19] Microinjection of the ras neutralizing antibody Y13–259 has been shown to block neurite outgrowth of PC-12 cells induced by nerve growth factor (NGF)[20]. This result was corroborated by experiments demonstrating that microinjection of GDPβS bound tumorigenic ras failed to induce neurite outgrowth.[21] In our studies, SCH-53870, when added to the cell culture medium, was found to inhibit NGF-stimulated neurite outgrowth in the 10–20 μM range. SCH-53239 and SCH-54292 showed no effect at this concentration range. Since ras nucleotide exchange is an intracellular process, the concentration of inhibitor within the cell is critical. Perhaps the intracellular drug concentration differences (not measured) may explain the observed potency diferences in the PC-12 cellular assay.

Conclusions

Our findings demonstrate the utility of molecular modeling, NMR and mass spectrometry in discovering ras nucleotide exchange inhibitors and in elucidating the binding mode in which one inhibitor interacts with the ras protein. This study has revealed a previously unknown binding site in the critical Switch-2 region of the ras protein that is capable of binding a small organic molecule. These discoveries should prove useful in understanding how intracellular components interact and control the function of ras, and may lead to other synthetic ras inhibitors. The spectroscopic techniques developed and reported in this communication are expected to provide critical information for

future drug discovery efforts, particularly when X-ray crystallographic determinations of protein–drug complexes are unavailable.

Experimental

General methods

All reagents were used without further purification. Melting points were determined using an Electrothermal Digital Melting Point apparatus and are uncorrected. Elemental analyses were performed on either a Leeman CE 440 or a FISONS EA 1108 elemental analyzer. ^1H and ^{13}C NMR spectra were recorded on either a Varian VXR-200 (200 MHz) or Varian Gemini-300 (300 MHz) NMR spectrometers using Me_4Si as an internal standard. For ^{13}C NMR, a Nalorac Quad nuclei probe was used. ^1H NMR data were recorded using either a GE Omega 500 (500 MHz) or Varian Unity Plus 600 (600 MHz) NMR spectrometers. FT-IR spectra were recorded using a BOMEN Michelson 120 spectrometer. Mass Spectra were recorded using either EXTREL 401 (Chemical Ionization), JEOL or MAT-90 (FAB), VG ZAB-SE (SIMS), or SCIEX API III triple quadrupole (Electrospray) mass spectrometers. Optical rotations were recorded on a Perkin–Elmer 243B polarimeter. All calculations were performed on Silicon Graphics Indigo2 and Challenge XL computers. Monte Carlo calculations were performed using Macromodel 4.0 with AMBER potentials (5000 starting conformations, iteration limit = 1000, 0.001 kJ/mol Å convergence criteria). Geometric analysis of the intermolecular NOEs was performed using a program developed in-house for this purpose. Energy minimizations of the ras-GDP–SCH-54292 complex were carried out using DISCOVER with the cvff and AMBER forcefields.

4-Nitro-*N*,*N*-bis(2-hydroxyethyl)benzenesulfonamide (1). To a cooled (ice-water bath) soln of diethanolamine (10.4 g) and triethylamine (14 mL) in CH_2Cl_2 (250 mL) was added portion-wise a mixture of 4-nitrobenzenesulfonyl chloride (21.9 g) in CH_2Cl_2 (200 mL). The ice-water bath was removed and the reaction mixture was allowed to stir at room temperature overnight. The mixture was poured into a separatory funnel and shaken with aq 1 M HCl. The organic phase was sepd and washed with a satd aq soln of Na_2CO_3. The organic phase was concd in vacuo and dried under vacuum. Pure product: 17.4 g solid, 61% yield, mp 112–113 °C; ^1H NMR (300 MHz, DMSO-d_6): δ (ppm) 3.25 (t, 4H, J = 6 Hz), 3.51 (m, 4H), 4.84 (t, 2H, J = 5.3 Hz, exch D_2O), 8.09 (d, 2H, J = 8.3 Hz), 8.40 (d, 2H, J = 8.3 Hz); ^{13}C NMR (300 MHz, DMSO-d_6); δ (ppm) 50.62, 59.45, 124.45, 128.41, 144.85, 149.54; FAB-MS *m/z* 291 (MH^+, 100%). Anal. calcd for $C_{10}H_{14}N_2SO_6$: C, 41.38; H, 4.86; N, 9.65. Found: C, 40.77; H, 4.72; N, 9.31.

4-Nitro-*N*,*N*-bis[(2-methanesulfonyloxy)ethyl]benzenesulfonamide (2). To a cooled (ice-water bath) mixture of **1** (17.3 g) and triethylamine (21 mL) in

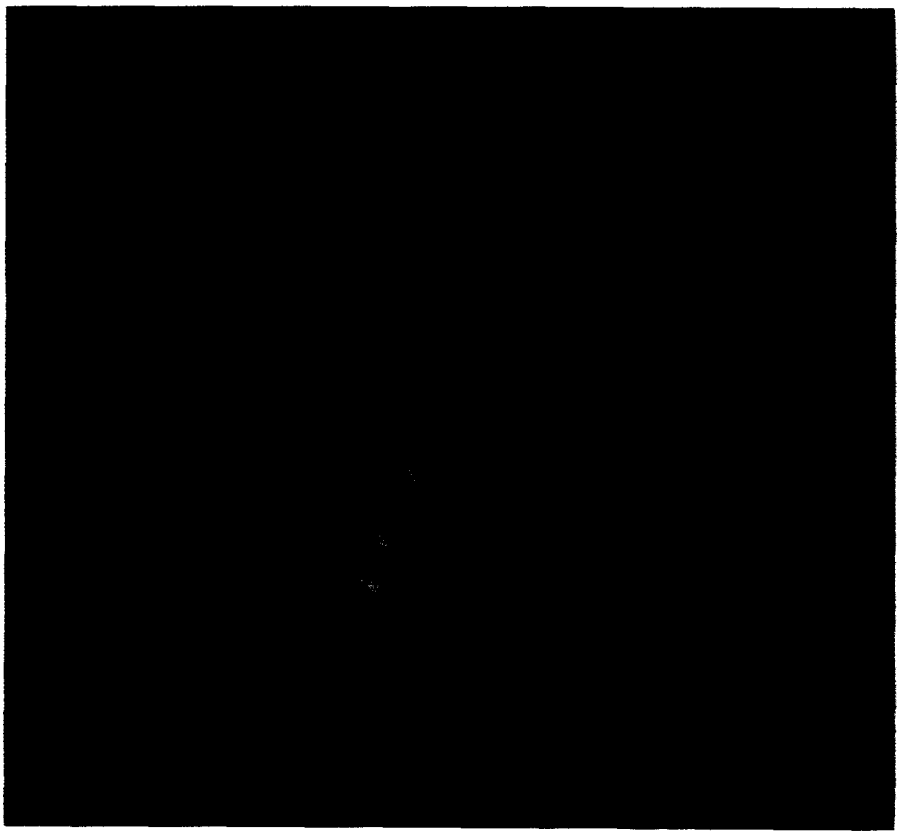


Figure 4. Proposed structure of the ras-GDP–SCH-54292 complex..

CH_2Cl_2 (500 mL) methanesulfonyl chloride (10.3 mL) was added drop-wise. The ice-water bath was removed and the reaction mixture was allowed to stir at room temperature for 48 h. The mixture was filtered and the solids were washed with CH_2Cl_2, and dried in vacuo. The filtrate was washed with water, dried over anhydrous $MgSO_4$ and concd in vacuo. Pure product: 17.1 g solid, 64% yield; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 3.22 (s, 6H), 3.66 (m, 4H, J=6 Hz), 4.35 (t, 4H, J=6 Hz), 8.19 (br d, 2H, J=8.3 Hz, 8.44 (br d, 2H, J=8.3 Hz); ^{13}C NMR (300 MHz, DMSO-d_6): δ (ppm) 36.49, 47.62, 66.79, 123.87, 127.96, 143.23; FAB-MS m/z 447 (MH^+, 79.31%), 469 (MNa^+, 100%). Anal. calcd for $C_{12}H_{18}N_2S_3O_{10}$: C, 32.28; H, 4.06; N, 6.27. Found: C, 32.17; H, 4.03; N, 6.17.

4-Nitro-*N*-[2-(2-naphthyloxy)ethyl]-*N*-[(2-methanesulfonyloxy)ethyl]benzenesulfonamide (3). To a solution of 2-naphthol (5.52 g) and KOH (2.15 g) in water (20 mL) was added **2** (17.1 g), acetone (500 mL) and *N*,*N*-dimethylformamide (30 mL). The resulting mixture was stirred at 75 °C for 15 h. The mixture was cooled to room temperature and concd in vacuo. The residue was dissolved in CH_2Cl_2 and water, shaken and the phases were separated. The organic phase was concentrated in vacuo and then purified by flash column chromatography (silica gel) using hexane:EtOAc (75%, 6L; 67%, 3 L; 50%, 8 L). Pure product: 10.1 g solid, 53% yield, mp 96.7–100.3 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 3.04 (s, 3H), 3.80 (m, 4H), 4.30 (t, 2H, J=5 Hz), 4.52 (t, 2H, J=5 Hz), 7.01 (dd, 1H, J=9, 2 Hz), 7.08 (d, 1H, J=2 Hz), 7.39 (dt, 1H, J=8, 1 Hz), 7.49 (dt, 1H, J=8, 1 Hz), 7.70–7.84 (m, 3H), 8.06 (br d, 2H, J=9 Hz), 8.32 (d, 2H, J=9 Hz); ^{13}C NMR (300 MHz, DMSO-d_6): δ (ppm) 37.23, 48.43, 48.71, 66.33, 67.29, 106.79, 118.16, 124.29, 124.60, 126.86, 127.80, 128.38, 128.51, 129.29, 129.86, 134.38, 145.00, 150.28, 155.84; FAB-MS-NBA m/z 495 (MH^+, 46.62%), 494 (100%), 493 (98.10), 351 (99.86), 307 (82.26), 289 (54.56). Anal. calcd for $C_{21}H_{22}N_2S_2O_8$: C, 50.95; H, 4.48; N, 5.66. Found: C, 50.67; H, 4.53; N, 5.66.

4-Nitro-*N*-[2-(2-naphthyloxy)ethyl]-*N*-[2-(2-amino-6-chloro-9*H*-purin-9-yl)ethyl]benzenesulfonamide (4). A mixture of **3** (5.50 g), 2-amino-6-chloropurine (1.87 g), K_2CO_3 (1.69 g) and *N*,*N*-dimethylformamide (90 mL) was stirred at 85 °C for 48 h. The mixture was cooled to room temperature, diluted with EtOAc, filtered, and the filtrate was concd in vacuo. The residue was purified by flash column chromatography (silica gel) using EtOAc:hexane (50%, 2 L; 67%, 1 L), then hexane:acetone (60%, 3 L). Pure product: 3.48 g solid, 55% yield, mp 191–193 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 3.69 (br t, 2H, J=5 Hz), 3.78 (br t, 2H, J=6 Hz), 4.20 (br t, 2H, J=5 Hz), 4.51 (br t, 2H, J=6 Hz), 5.1 (br m, 2H), 6.98 (dd, 1H, J=9, 2 Hz), 6.99 (overlapping s, 1H), 7.31–7.53 (m, 2H), 7.68–7.82 (m, 3H), 7.88 (s, 1H), 7.97 (d, 2H, J=9 Hz), 8.28 (d, 2H, J=9 Hz); ^{13}C NMR (300 MHz, DMSO-d_6): δ (ppm) 41.09, 46.51, 46.91, 65.51, 106.71, 118.18, 123.03, 123.64, 124.21, 124.70, 126.35, 126.56, 127.39, 128.02, 128.46, 129.18, 133.98, 142.97, 144.37, 149.08, 149.20, 153.96, 155.49, 159.58; FAB-MS m/z 568 (MH^+, 71.05%), 569 (43.05%), 570 (49.53). Anal. calcd for $C_{25}H_{22}N_7SO_5Cl$: C, 52.86; H, 3.90; N, 17.26. Found: C, 52.17; H, 3.89; N, 17.26.

4-Nitro-*N*-[2-(2-naphthyloxy)ethyl]-*N*-[2-(2-amino-1,9-dihydro-6-oxo-6*H*-purin-9-yl)ethyl]-benzenesulfonamide (5). To a soln of **4** (2.2 g) in *N*,*N*-dimethylformamide (40 mL) and MeOH (10 mL) was added a soln of concd HCl:HOAc (1:1, 75 mL) and the resultant mixture was stirred at room temperature for 72 h. The mixture was concd in vacuo, diluted with CH_2Cl_2 (200 mL) and 1N HCl and the mixture was stirred vigorously for 1 h, then filtered. The yellow solid was washed with water and dried under vacuum. Pure product: 1.91 g solid, 90% yield, mp 295.2–296.6 °C; ^{1}H NMR (200 MHz, DMSO) d (ppm) 3.78 (m, 4H), 4.21 (m, 4H), 6.43 (br s, 2H, exchangeable with D_2O), 7.04 (dd, 1H, J=9, 2 Hz), 7.25 (br s, 1H), 7.36 (t, 1H, J=8 Hz), 7.48 (t, 1H, J=8 Hz), 7.66 (s, 1H), 7.82 (m, 3H), 8.00 (d, 2H, J=9 Hz), 8.25 (d, 2H, J=9 Hz), 10.51 (s, 1H); FAB-MS m/z 550 (MH^+, 100%). Anal. calcd for $C_{25}H_{23}N_7SO_6$: C, 54.64; H, 4.22; N, 17.84. Found: C, 53.98; H, 4.21; N, 17.19.

4-Hydroxylamino-*N*-[2-(2-naphthyloxy)ethyl]-*N*-[2-(2-amino-1,9-dihydro-6-oxo-6*H*-purin-9-yl)ethyl]benzenesulfonamide (SCH-53239). A mixture of **5** (58 mg) and 10% Pd on C (10.5 mg) in *N*,*N*-dimethylformamide (30 mL) was shaken at 60 psi with H_2 in a parr hydrogenator for 6 h. Filtration and concn of the soln in vacuo afforded a residue which was triturated with hot MeOH:acetone to afford a light yellow solid: 52 mg, 92% yield; ^{1}H NMR (200 MHz, DMSO): δ (ppm) 3.50 (m, 4H), 4.09 (m, 2H), 4.21 (m, 2H), 6.46 (br s, 2H, exchangeable with D_2O), 6.90 (d, 2H, J=8 Hz), 7.12 (dd, 1H, J=9, 2 Hz), 7.23 (br s, 1H), 7.38 (t, 1H, J=8 Hz), 7.49 (t, 1H, J=8 Hz), 7.63 (d, 2H, J=8 Hz), 7.65 (overlapping s, 1H), 7.83 (m, 3H), 8.74 (s, 1H, exchangeable with D_2O), 9.09 (s, 1H, exchangeable with D_2O), 10.58 (s, 1H, exchangeable with D_2O); ^{13}C NMR (300 MHz, DMSO-d_6): δ (ppm) 41.94, 47.90, 48.1, 66.11, 106.77, 111.83, 116.59, 118.56, 123.99, 126.71, 127.02, 127.71, 128.78, 129.67, 134.40, 137.94, 151.58, 153.64, 155.86, 156.07, 157.11; FAB-MS m/z 536 (MH^+, 100%), 535 (64.46%). Anal. calcd for $C_{25}H_{25}N_7SO_5$: C, 56.07; H, 4.71; N, 18.31. Found: C, 55.71; H, 4.71; N, 17.60.

4-Nitro-*N*-[2-(2-naphthyloxy)ethyl]benzenesulfonamide (7). To a cooled (ice-water bath) suspension of naphthyloxyethylamine hydrochloride[5] (**6**, 39.4 g) and triethylamine (66 mL) in CH_2Cl_2 (700 mL) was added portion-wise 4-nitrobenzenesulfonyl chloride (43.3 g of 90% purity). The ice-water bath was removed and the reaction mixture was allowed to stir at room temperature overnight. The mixture was poured into a separatory funnel and shaken with a satd aq soln of Na_2CO_3. The aq phase was removed and the organic phase was washed with 1 M hydrochloric acid, dried over

anhydrous $MgSO_4$, filtered and concd in vacuo. The residue was purified by flash column chromatography (silica gel) using 25% EtOAc:hexane (30 L). Pure product: 13.5 g, 21% yield; solid, mp 132.7–134.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 3.35 (m, 2H), 4.09 (t, 2H, J=6 Hz), 7.00 (dd, 1H, J=9, 2 Hz), 7.21 (d, 1H, J=2 Hz), 7.35 (dt, 1H, J=8, 1 Hz), 7.46 (dt, 1H, J=8, 1 Hz), 7.72–7.88 (m, 3H), 8.10 (d, 2H, J=9 Hz), 8.36 (d, 2H, J=9 Hz), 8.45 (t, 1H, J=6 Hz); ^{13}C NMR (300 MHz, DMSO-d_6): δ (ppm) 42.01, 66.23, 106.57, 118.35, 123.57, 124.42, 126.31, 126.53, 127.38, 127.9, 128.40, 129.08, 133.99, 146.36, 149.27, 155.72; FAB-MS-NBA *m/z* 395 (MNa^+, 71.16%), 373 (MH^+, 75.27%), 372 (M^+, 92.67%), 229 (100.0%). Anal. calcd for $C_{18}H_{16}N_2SO_5 \cdot H_2O$: C, 55.38; H, 4.65; N, 7.18. Found: C, 55.74; H, 4.26; N, 7.61.

4-Hydroxylamino-*N*-[2-(2-naphthyloxy)ethyl]benzenesulfonamide (SCH-53870). A stirred soln of nitro compound **7** (2.98 g, 8.0 mmol) dissolved in anhydrous tetrahydrofuran (50 mL) and absolute EtOH (50 mL) under nitrogen atmosphere was cooled in ice-water. 5% Pd on C catalyst was added (0.30 g) and after 15 min, hydrazine monohydrate (0.78 mL, 16.0 mmol) was added dropwise to the cooled soln while maintaining vigorous stirring under nitrogen atmosphere. After 1.5 h of stirring at 0 °C, the reaction mixture was filtered through a pad of celite under N_2 and the solids washed with acetone. The filtrate was conced in vacuo. Re-crystallization of the resulting solid from EtOAc:hexane afforded the title compound. Pure product: 2.7 g, 94% yield; white solid, mp 255 °C (dec); ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 3.15 (m, 2H), 4.10 (t, 2H, J=5 Hz), 6.92 (d, 2H, J=9 Hz), 7.13 (dd, 1H, J=9, 2 Hz), 7.28 (d, 1H, J=2 Hz), 7.36 (br t, 1H, J=7.5 Hz), 7.48 (br t, 1H, J=7.5 Hz), 7.65 (d, 2H, J=9 Hz), 7.83 (m, 3H), 8.70 (s, 1H), 8.99 (s, 1H); ^{13}C NMR (300 MHz, DMSO-d_6): δ (ppm) 41.85, 66.49, 106.67, 111.47, 112.65, 118.62, 123.62, 126.38, 126.67, 127.47, 127.90, 128.49, 129.05, 129.27, 134.16, 155.15, 156.06; IR (Nujol) 3300, 2980, 2940, 2860, 1625, 1595, 1460, 1380, 1300, 1260, 1220, 1150, 1080, 1045, 955, 840, 800, 720, 700 cm^{-1}; SI-MS *m/z* 359 (MH^+, 55%), 257 (100%). Anal. calcd for $C_{18}H_{18}N_2SO_4$: C, 60.32; H, 5.06; N, 7.82; S, 8.94. Found: C, 61.01; H, 4.84; N, 7.86; S, 9.23.

4-Nitro-*N*-[2-(2-naphthyloxy)ethyl]-*N*-[2-deoxy-tetra-acetyl-β-D-glucos-2-yl]benzene-sulfonamide (8) and 4-nitro-*N*-[2-(2-naphthyloxy)ethyl]-*N*-[2-deoxy-tetra-acetyl-α-D-glucos-2-yl]benzene-sulfonamide. To a solution of nitrobenzenesulfonamide (**7**) (15.6 g, 41.9 mmol) dissolved in anhydrous dimethylformamide (200 mL) was added solid NaH (7.43 g, 60% in mineral oil) portionwise. Acetobromo-α-D-glucose (35.6 g, 86.6 mmol) was added in one portion and after stirring for 24 h at room temperature under nitrogen, an additional 10.4 g of acetobromo-α-D-glucose and 30 mL DMF were added. After 20 days, the reaction mixture was concentrated in vacuo, the residue diluted with CH_2Cl_2 (500 mL) and water (100 mL), and the soln acidified with 1 M HCl (300 mL). The mixture was shaken well and the organic phase was sepd, dried over anhydrous $MgSO_4$, filtered, and concd in vacuo. Purification of the residue by flash column chromatography (silica gel) using hexane:EtOAc (100%, 1.5 L; 80%, 6 L; 75%, 6 L; 67%, 10 L; 50%, 8 L; 25%, 2 L; 20%, 5 L) provided a sticky foam. Dissolution of this foam in hot MeOH and stirring the resulting soln vigorously resulted in precipitation of the product which was filtered off and dried under vacuum. Pure product: 12.7 g, 43% yield; yellow solid, mp 64.5–66.4 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 2:1 mixture of diastereomers): δ (ppm) 1.83 (s, 3H), 2.01 (s, 3H), 2.04 (s, 3H), 2.06 (s, 3H), 3.48–3.68 (m, 1H), 3.73–3.92 (m, 2H), 4.10 (m, 2H), 4.19 (m, 2H), 5.08 (t, 1H, J=9.5 Hz), 5.27–5.38 (m, 2H), 5.46 (m, 1H), 6.94 (dd, 1H, J=9, 2.5 Hz), 7.00 (d, 1H, J=2.5 Hz), 7.34 (br t, 1H, J=7 Hz), 7.45 (br t, 1H, J=7 Hz), 7.65–7.8 (m, 3H), 8.09 (br d, 2H, J=9 Hz), 8.28 (br d, 2H, J=9 Hz); ^{13}C NMR (300 MHz, DMSO-d_6, *mixture of diastereomers):* δ (ppm) 20.22, 20.25, 20.37, 20.42, 20.49, 42.40, 60.39, 61.56, 65.19, 66.15, 66.91, 67.43, 68.08, 72.22, 72.66, 73.46, 83.68, 106.52, 116.32, 123.64, 124.26, 124.41, 126.39, 126.58, 126.64, 127.45, 128.47, 128.88, 129.09, 129.16, 134.06, 139.08, 144.66, 149.83, 155.69, 169.09, 169.28, 169.33, 169.56, 169.69, 169.82, 169.95; IR (Nujol) 2930, 2745, 2685, 1755, 1530, 1460, 1380, 1310, 1155, 1030, 720 cm^{-1}; SI-MS *m/z* 702 (M^+, 43%), 331 (94%), 257 (100%); $[\alpha]_D^{25}$ −34.2 ° *c* 0.5 (DMSO). Anal. calcd for $C_{32}H_{34}N_2SO_{14} \cdot H_2O$: C, 53.33; H, 5.04; N, 3.89. Found: C, 52.90; H, 4.92; N, 4.23.

4-Nitro-*N*-[2-(2-naphthyloxy)ethyl]-*N*-[2-deoxy-β-D-glucos-2-yl]benzenesulfonamide (9). The diasteriomeric mixture of nitro compound **8** and its α-anomer (0.42 g, 0.60 mmol), anhydrous MeOH (20 mL), K_2CO_3 (0.33 g, 2.4 mmol) and distilled water (5 mL) was stirred at room temperature. After 2 days, the resulting mixture was filtered and the filtrate discarded. The solid was boiled in MeOH, refiltered while hot through a fritted glass filter, and the filtrate was allowed to cool to room temperature slowly. The resulting precipitate was filtered and dried under vacuum and shown to be only the β-anomer.[22] Pure product: 0.21 g, 67% yield; solid, mp 237.5–238 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 3.13 (m, 1H), 3.23–3.52 (m, 5H), 3.61 (m, 2H), 4.28 (m, 2H), 4.48 (m, 1H, exchangeable with D_2O), 5.00 (br d, 1H, J=7.5 Hz), 5.08 (d, 1H, J=5 Hz, exchangeable with D_2O), 5.26 (m, 1H, exchangeable with D_2O), 5.43 (m, 1H, exchangeable with D_2O), 7.05 (dd, 1H, J=9, 2.5 Hz), 7.27 (br s, 1H), 7.38 (br t, 1H, J=8 Hz), 7.49 (br t, 1H, J=8 Hz), 7.82 (m, 3H), 8.30 (m, 4H); ^{13}C NMR (300 MHz, DMSO-d_6): δ (ppm) 40.52, 59.31, 65.10, 68.10, 68.68, 75.97, 77.47, 85.70, 105.51, 117.20, 122.62, 123.01, 125.34, 125.46, 126.31, 127.33, 128.18, 132.87, 143.57, 148.49, 154.42; IR (Nujol) 3540, 3470 (br), 2930, 1520, 1460, 1380, 1315, 1280, 1220, 1170, 1085, 970, 952, 850, 720 cm^{-1}; FAB-MS *m/z* 534 (M^+, 35%), 557 (MNa^+, 44%), 373 (100%); $[\alpha]_D^{25}$ −31.0 °, *c* 0.5 (DMSO). Anal. calcd for $C_{24}H_{26}N_2SO_{10}$: C, 53.93; H, 4.90; N, 5.24. Found: C, 53.90; H, 4.96; N, 4.94.

4-Hydroxylamino-*N*-[2-(2-naphthyloxy)ethyl]-*N*-[2-deoxy-β-D-glucos-2-yl]benzenesulfonamide (SCH-54292). A stirred soln of nitro compound **9** (3.81 g, 7.13 mmol) dissolved in anhydrous tetrahydrofuran (300 mL), absolute ethanol (250 mL) and anhydrous dimethylformamide (200 mL) under nitrogen atmosphere was cooled in ice-water. 5% Pd on C catalyst was added (0.39 g) and after 30 min, hydrazine monohydrate (0.35 mL, 7.13 mmol) was added dropwise to the cooled soln while maintaining vigorous stirring under nitrogen atmosphere. The progression of the reaction was followed by analytical thin layer chromatography (TLC). After several hours of stirring at 0 °C, additional hydrazine monohydrate was added dropwise since starting nitro compound **11** was still present (as determined by TLC). After stirring for several more hours at 0 °C, the reaction mixture was filtered through a pad of celite under N_2 and the solids were washed with acetone. The filtrate was concd in vacuo, then the resulting solid was re-dissolved in acetone (15 mL) and slowly poured into distilled water (200 mL). After several minutes, the resultant precipitate was filtered through a fritted glass funnel and dried under nitrogen (2.1 g). The filtrate was concd in vacuo and the residue was purified by flash column chromatography (silica gel) using MeOH:CH_2Cl_2 (5–10%, 8 L) to provide an additional amount of pure product (1.2 g). Pure product: 3.3 g, 89% yield; white solid, mp 119.7–120.5 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 3.03–3.70 (m, 7H in D_2O:DMSO-d_6), 4.05 (m, 1H), 4.26 (m, 2H), 4.94 (m, 1H), 6.86 (m, 2H), 7.12 (br d, 1H), 7.30 (br s, 1H), 7.37 (m, 1H), 7.48 (m, 1H), 7.79 (m, 5H), 8.72 (br s, 1H, exchangeable with D_2O), 9.02 (br m, 1H, exchangeable with D_2O); ^{13}C NMR (300 MHz, DMSO-d_6): δ (ppm) 40.80, 60.40, 66.23, 69.14, 70.07, 77.23, 78.33, 86.74, 106.57, 111.29, 118.43, 123.78, 126.55, 126.62, 127.41, 127.50, 128.49, 129.23, 129.43, 134.08, 155.39, 155.69; IR (Nujol) 3350 (br), 2930, 1720, 1632, 1597, 1460, 1380, 1260, 1217, 1160, 1075, 1030, 825, 720 cm^{-1}; FAB-MS *m/z* 521 (MH^+, 21%), 543 (MNa^+, 100%); $[\alpha]_D^{25\,°C}$ −42.6 °, *c* 0.5 (DMSO). Anal. calcd for $C_{24}H_{28}N_2SO_9$: C, 55.38; H, 5.42; N, 5.38. Found: C, 55.57; H, 5.99; N, 4.75.

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(*Received in U.S.A.* 3 *March* 1996; *accepted* 2 *May* 1996)