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Sugar-Derived Ras Inhibitors: Group Epitope Mapping by NMR Spectroscopy and Biological Evaluation

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Novel inhibitors of Ras protein activation have been found, containing a bicyclic core derived from D-arabinose and benzyl and phenylhydroxylamine moieties. NMR studies (trNOE, saturation-transfer difference, STD) of the binding between these molecules and human p21 h-Ras are reported. A pharmacophore mapping indicates that both the benzyl and the phenylhydroxylamine moieties are essential for protein binding. Molecules lacking one of these groups were synthesized and tested to confirm this hypothesis, and no interaction with Ras in vitro, nor biological activity in mammalian cells was observed. Our studies led to the development of molecules that selectively inhibit Ras-dependent cellular growth in mammalian cells.

Introduction

Mutated forms of the Ras proteins are present in about 30% of human tumours.[1] Point mutations of Ras (k-Ras) generate constitutively active proteins responsible for uncontrolled cellular growth and proliferation leading to oncogenesis. Ras activation is also known to be involved in cancer cell survival after radiation or chemotherapy in a number of different tumour types, thus contributing to tumour re-insurgence after therapy.[2] There is, therefore, a great deal of interest in the search for selective inhibitors of Ras activation as potential anticancer drugs. In this context, we have prepared and tested novel molecules (compounds **1**–**4**, Figure 1) that are capable of inhibiting nucleotide exchange and guanosine diphosphate (GDP) dissociation from human p21 h-Ras, and of blocking growth and proliferation on normal and k-Ras-transformed mammalian cells.[3] Interestingly, the inhibition effect of molecules **2** and **4** on cellular growth and proliferation of k-Ras-transformed mouse fibroblasts were more pronounced than on normal cells, thus indicating a specificity action toward Ras-mediated signalling.

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Inhibitors **1**–**4** (Figure 1) present two benzyl groups and a phenylhydroxylamino group linked to a rigid scaffold. We previously reported on the conformational properties of the bicyclic core derived from the natural monosaccharide D-arabinose:[4] it presents the proper spatial disposition to accommodate pharmacophore groups for interaction with Ras protein. According to molecular modelling and docking studies, compounds **1**–**4** are all able to bind Ras with the orientations of the aromatic and phenylhydroxylamine moieties similar to those adopted by a number of inhibitors developed by Schering-Plough.[5] All inhibitors seem to adopt a similar bound conformation despite the different configurations of the C-2 atoms of the bicycles and the different natures (amide or sulfonamide) of the C-2 linkers. This hypothesis is supported by the experimental observation that all the molecules show similar affinities for Ras, and inhibit with very similar potency both guanine nucleotide exchange and GDP dissociation from p21 h-Ras. Docking analysis, in agreement with data previously reported by Schering-Plough,[6] points out that the phenylhydroxylamine moiety of these inhibitors plays an important role in the interaction with the protein. The hydroxylamine group binds in all cases with the Mg^{++} ion that is also coordinated by the β-phosphate group of GDP and interacts through hydrogen bonds with Thr58. We also found important interactions between the amide or sulfonamide groups of the inhibitors and Ras residues Gly10/Gly60. The benzyl ether on C-4 is placed in a hydrophobic cavity of Ras and interacts with residues Val9, Met72, Gln99 and Ile100. On the contrary, the benzyl ether on C-2′ points outward from the binding site and has no important interactions with Ras (Figure 2).

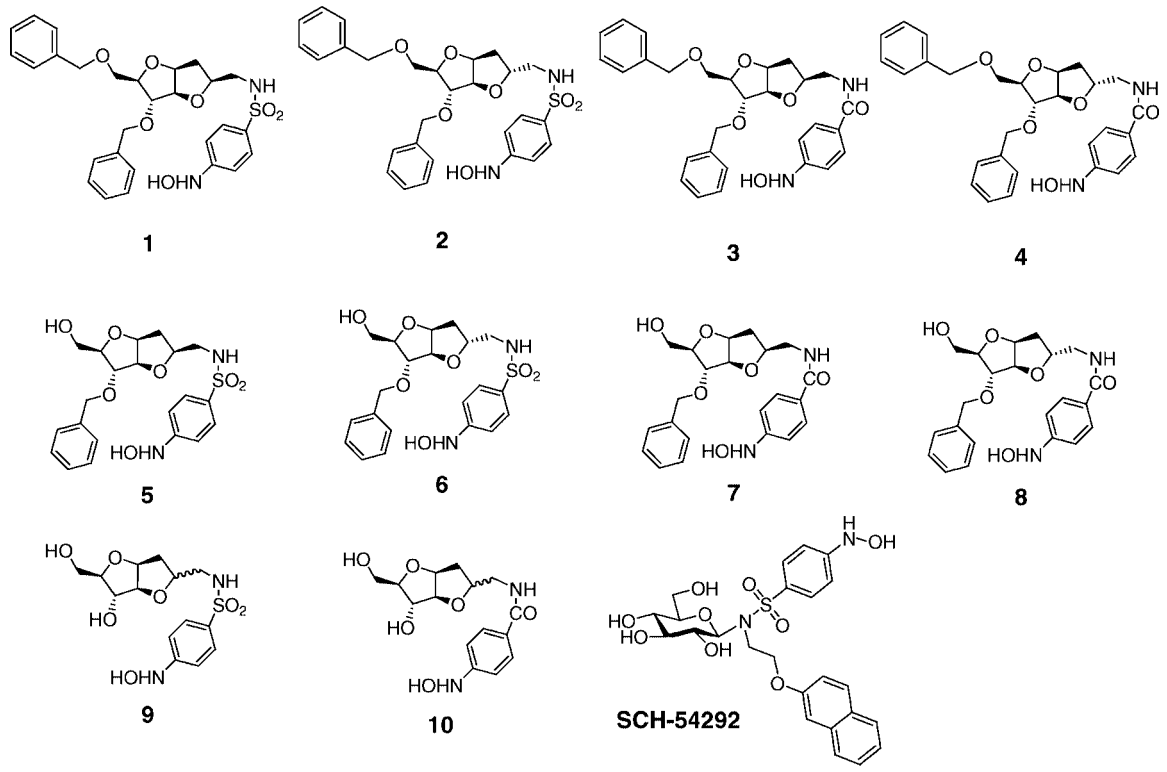


Figure 1. Chemical structures of compounds **1**–**10** and **SCH-54292**.

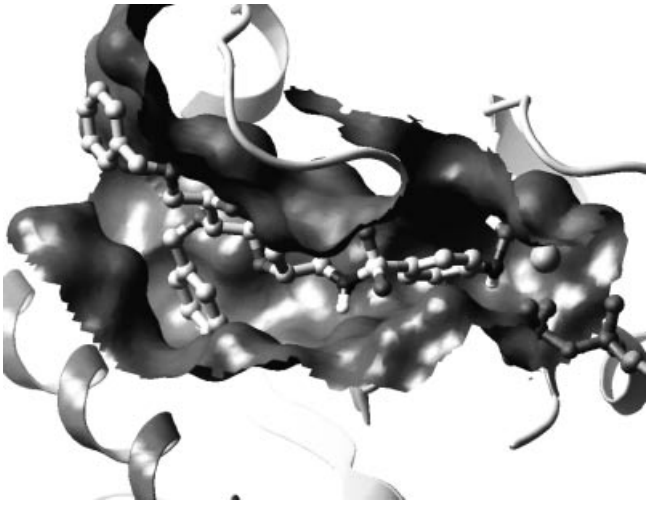


Figure 2. Compound **1** docked into the binding cavity of Ras using the GLIDE program (*GLIDE* 2.7, Schrodinger LLC, **2003**). While the benzyl group on C-4 of inhibitor is inserted into a binding pocket, the benzyl group on C-2′ protrudes from the protein.

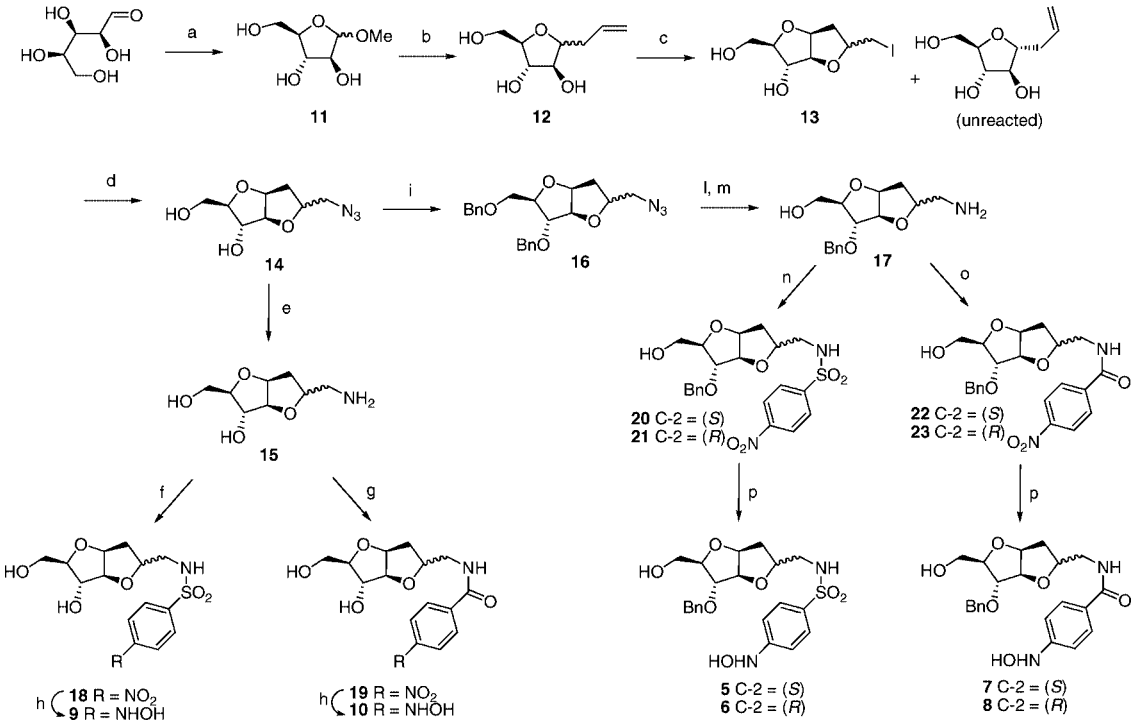
We present here a second generation of inhibitors, namely molecules **5**–**10**, structurally very similar to the previously tested compounds **1**–**4**. Compounds **5**–**8** have a free hydroxy group on C-2′ instead of the benzyl group, **9** and **10** have free hydroxy groups on both C-4 and C-2′. These molecules have been designed for two main reasons: to investigate the importance of the aromatic (benzyl) rings in the interaction with Ras, and to have greater water solubility compared to compounds **1**–**4**. The inhibitor **SCH-54292** was also prepared by optimizing the synthetic procedure previously adopted by Schering. This compound was used as a reference in NMR binding studies and biological tests. The comparison of the binding properties and biological activities of compounds **1**–**10** will provide further important information about the structure-activity relationship in this family of inhibitors.

Results

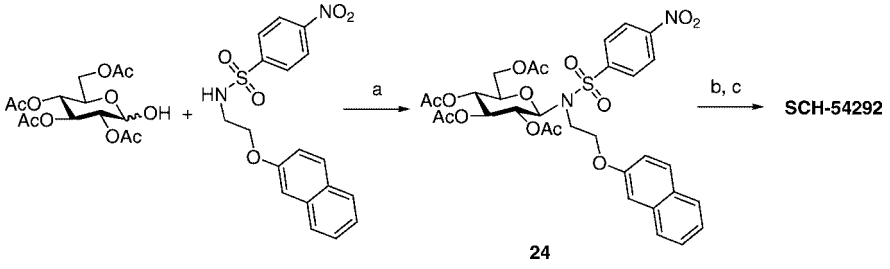
Synthesis

Compounds **5**–**10** were prepared according to the synthetic pathway of Scheme 1. The common bicyclic iodide intermediate **13** was obtained by the iodo-promoted cyclisation of the allyl-C-arabinofuranoside **12**. Only the β-anomer reacted, and the α-anomer was recovered as reported elsewhere.[3,4] Nucleophilic displacement of iodine with tetrabutylammonium azide afforded **14**, which was converted into the corresponding amine **15** by treatment with triphenylphosphane. Reaction of **15** with *p*-nitrobenzenesulfonyl chloride, followed by reduction of the aromatic nitro group with hydrazine and Pd/C, yielded compound **9** as a mixture of diastereoisomers. Alternatively, the condensation of **15** with *p*-nitrobenzoic acid in the presence of *N*,*N*-diisopropylcarbodiimide (DIC) and *N*-hydroxybenzotriazole (HOBt), followed by reduction with hydrazine and Pd/C, afforded the diastereomeric mixture **10**.

Intermediate **14** was *O*-benzylated by using benzyl bromide and sodium hydride to obtain **16**. The C-2′ benzyl ether of **16** was selectively converted into an *O*-acetate by treatment with Ac_2O/TFA, and the acetyl group was then



Scheme 1. Reagents and conditions: a) AcCl, MeOH, 18 h, 91%; b) BTSFA, CH_3CN, 100 °C, 3 h, then ATMS, TMSOTf, CH_3CN, room temperature, 1 h, then H_2O, 97%; c) NIS, THF, 90 °C, 10 min, 58%; d) Bu_4NN_3, DMF, 70 °C, 72 h, 90%; e) PPh_3, THF/H_2O, 70 °C, 14 h, 85%; f) *p*-nitrobenzenesulfonyl chloride, pyridine, 0 °C, 6 h, 64%; g) DIC, HOBt, DIPEA, *p*-nitrobenzoic acid, DMF, room temperature, 24 h, 75.5%; h) NH_2NH_2, Pd/C, THF, 0 °C, 45 min, 98%; i) NaH, BnBr, DMF, room temperature, 15 min, 91%; l) Ac_2O/TFA (4:1, v/v), room temperature, 90 min, then MeONa, MeOH, room temperature, 30 min, 85%; m) PPh_3, THF/H_2O, 70 °C, 14 h, 94%; n) TEA, *p*-nitrobenzenesulfonyl chloride, CH_2Cl_2, 0 °C, 5 h, 82%; o) DIC, HOBt, DIPEA, *p*-nitrobenzoic acid, DMF, room temperature, 24 h, 70%; p) NH_2NH_2, Pd/C, THF, 0 °C, 45 min, 92%. BSTFA = *N*,*O*-bis(trimethylsilyl)trifluoroacetamide, ATMS = allyltrimethylsilane, TMSOTf = trimethylsilyl trifluoromethanesulfonate, NIS = *N*-iodosuccinimide, TEA = triethylamine, DIC = *N*,*N*-diisopropylcarbodiimide, HOBt = *N*-hydroxybenzotriazole, DIPEA = diisopropylethylamine.



Scheme 2. Reagents and conditions: a) PPh_3, DIAD, –80 °C, 1 h, then room temperature, 18 h, 84%; b) K_2CO_3, dry CH_3OH, 24 h, 95%; c) NH_2NH_2, Pd/C, THF, 0 °C, 45 min, 93%. DIAD = diisopropyl azodicarboxylate.

removed with sodium methoxide in methanol. The azide was reduced using triphenylphosphane in THF/water, affording amine **17**. Reaction of the amino group of **17** with *p*-nitrobenzenesulfonyl chloride allowed the introduction of the *p*-nitrophenylsulfonamide moiety (**20** and **21**). Alternatively, condensation with *p*-nitrobenzoic acid yielded amides **22** and **23**. Amide and sulfonamide diastereomers were separated at this point of the synthesis by flash chromatography on silica gel. Finally, reduction of the aromatic nitro group with hydrazine and Pd/C led to the final phenylhydroxylamine compounds **5**–**8**.

For the synthesis of compound **SCH-54292**, the critical *N*-glycosylation step of tetraacetylglucose with 4-nitro-*N*-[2-(2-naphthyloxy)ethyl]benzenesulfonamide was achieved by a Mitsunobu-type glycosylation (Scheme 2).[7] Unlike previously published methods[5] based on the use of tetraacetylbromoglucose as the glycosyl donor, this glycosylation reaction was efficient and almost totally stereoselective in favour of the β-*N*-glycoside, yielding **24** in 84% yield. Removal of the acetyl protecting groups was accomplished by mild basic hydrolysis (Na_2CO_3 in MeOH) in order to avoid base-promoted cleavage of the N–glycoside bond. The final compound **SCH-54292** was obtained after reduction of the nitro group with hydrazine and Pd/C.

Ras–Ligand Binding Studies by NMR

For ligands that exchange between the free and bound states at a moderately fast rate, trNOESY experiments pro-

vide a helpful means to study their conformations at the receptor binding sites. This is usually the case for carbohydrates (or glycomimetics) when they are bound to their receptors.[8] trNOESY experiments were performed to investigate the interactions in solution between compounds **5**–**10** and the Ras-GDP complex. p21 h-Ras was incubated in a [D_{11}]-Tris buffer, containing 10% CD_3OD, 100 mM NaCl, 5 mM $MgCl_2$, an amount of GDP equimolar to the

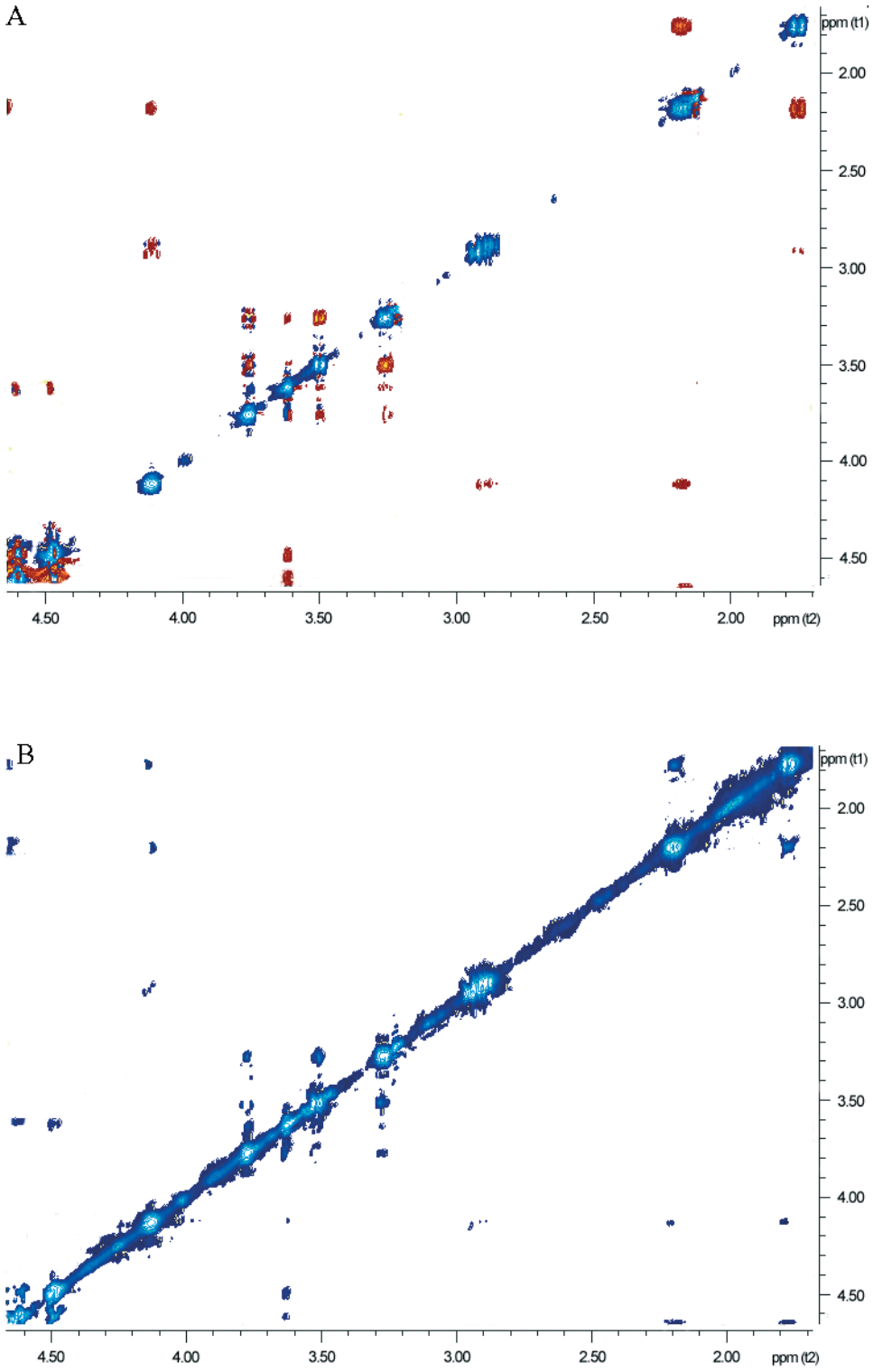


Figure 3. A) NOESY spectrum of compound **5**, mixing time = 800 ms; B) trNOESY spectrum of compound **5**-Ras-GDP mixture, mixing time = 200 ms. The spectral region between δ = 1.7 and 4.7 ppm, which contains all signals of protons on the bicyclic scaffold, is shown. Both the samples were dissolved in a [D_{11}]-Tris buffer at pH = 7.3, containing 10% CD_3OD, 100 mM NaCl, and 5 mM $MgCl_2$. Total sample volume was 450 μL.

Table 1. Selective T_1 and $T_{1\rho}$ values for compounds **5**–**8**.

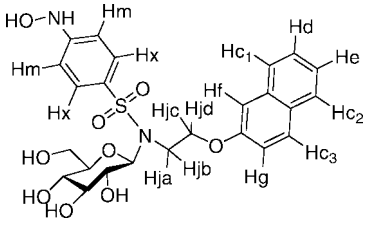
Sample	Hx	Hbenz	Hm	H-2	H-1
	Selective T_1				
5	2.07±0.02	2.37±0.01	2.32±0.01	1.14±0.01	0.58±0.01
5+Ras-GDP	0.76±0.03	1.08±0.01	0.96±0.03	0.54±0.01	0.32±0.01
6	2.17±0.01	2.48±0.01	2.27±0.02	1.22±0.01	0.50±0.01
6+Ras-GDP	0.75±0.02	0.95±0.01	0.77±0.01	0.59±0.01	0.31±0.01
7	2.11±0.01	2.46±0.02	2.38±0.02	1.01±0.01	0.58±0.01
7+Ras-GDP	0.81±0.02	0.94±0.03	0.83±0.03	0.45±0.01	0.32±0.02
8	2.45±0.03	2.75±0.04	2.56±0.05	1.13±0.01	0.57±0.01
8+Ras-GDP	1.30±0.02	1.59±0.03	1.30±0.01	0.76±0.01	0.31±0.02
	$T_{1\rho}$				
5	0.95±0.01	1.16±0.01	1.06±0.02	0.82±0.03	0.26±0.01
5+Ras-GDP	0.51±0.02	0.49±0.01	0.42±0.02	0.31±0.01	0.14±0.01
6	1.11±0.01	1.63±0.01	1.35±0.01	0.78±0.02	0.26±0.01
6+Ras-GDP	0.55±0.01	0.39±0.01	0.41±0.01	0.25±0.01	0.19±0.02
7	1.20±0.00	1.72±0.01	1.37±0.01	0.72±0.01	0.27±0.00
7+Ras-GDP	0.47±0.01	0.42±0.01	0.46±0.02	0.26±0.01	0.16±0.02
8	1.13±0.02	2.09±0.03	1.55±0.02	0.53±0.03	–
8+Ras-GDP	0.72±0.02	0.75±0.01	0.79±0.04	0.43±0.02	–

protein, and compounds **5**–**10**. An optimized ligand/protein molar ratio of 20 was used in all experiments. NOESY spectra of compounds **5**–**10** in the free state were obtained with mixing times of 800 ms. In trNOESY experiments, the optimal value of 200 ms for the mixing time was used. In contrast with what was observed in the free state, negative NOEs (trNOEs) were observed for molecules **5**–**8** as a consequence and proof of interaction with the protein (Figure 3). Under the same experimental conditions, compounds **9** and **10** did not show any trNOE signals, probably indicating their lack of interaction with the Ras-GDP complex. The specific binding of all new compounds to the Ras-GDP complex was also investigated by the analysis of the variations of the selective T_1 and $T_{1\rho}$ of several ligand protons measured in the free state and in the presence of the Ras-GDP complex (Table 1).

Selective T_1 and $T_{1\rho}$ values of free compounds **5**–**8** were significantly larger than those determined in the presence of the protein. This effect was evident in all protons analyzed, and it was particularly strong for the aromatic ones. As also observed in the trNOESY experiments (see above), the measurements of the selective T_1 and $T_{1\rho}$ values for compounds **9** and **10** provided values with no significant variations in the absence or presence of the Ras-GDP complex. For the reference compound, **SCH-54292**, the aromatic protons showed a decrease in selective T_1 and $T_{1\rho}$ values when passing from the free to the bound state, while no change of T_1 values was observed for protons of the sugar moiety (Table 2). Interestingly, the magnitude of the variations of both selective T_1 and $T_{1\rho}$ values was always much larger for compounds **5**–**8** than for **SCH-54292**.

Saturation-transfer difference (STD) experiments[9] are a very helpful means to detect ligand binding to receptors; therefore STD experiments were performed on the same ligand/protein mixtures with the aim of confirming the interaction, and of studying which region of the ligand directly interacts with Ras (epitope mapping).[9] The comparison

Table 2. Selective T_1 and $T_{1\rho}$ values for **SCH-54292**.



Sample	Hm	Hf	H-2
		T_1	
SCH-54292	2.52±0.08	1.24±0.05	–
SCH-54292+Ras-GDP	1.97±0.05	0.96±0.03	–
		$T_{1\rho}$	
SCH-54292	1.64±0.08	0.97±0.04	0.60±0.06
SCH-54292+Ras-GDP	1.33±0.04	0.62±0.07	0.60±0.06

between the ^{1}H spectra and STD spectra of compounds **5**–**8** (Figure 4) pointed out that the major interactions with Ras involved both the benzyl and the phenylhydroxylamine moieties of the ligands. In particular, the signals of the Hm and Hx (phenylhydroxylamine) and of the Ha and Hb protons (benzyl) were clearly observed in all STD experiments. In analogy with the previously described experiments, the STD experiments recorded for **9** and **10** did not show any signals. Moreover, the STD spectrum of the reference compound **SCH-54292** indicated the interaction of both naphthyl and phenylhydroxylamine groups with the protein, while no interaction with the sugar moiety was detected. As outlined in previous studies,[6] in this case the glucose portion functions to increase water solubility and does not play any role in the binding with Ras. Thus, all the NMR spectroscopic data for **5**–**8** and the parent compound seem to indicate major interactions of the aromatic residues with Ras, while the bicyclic moiety acts as scaffold to provide the proper orientation of the interacting residues, along with the required solubility.

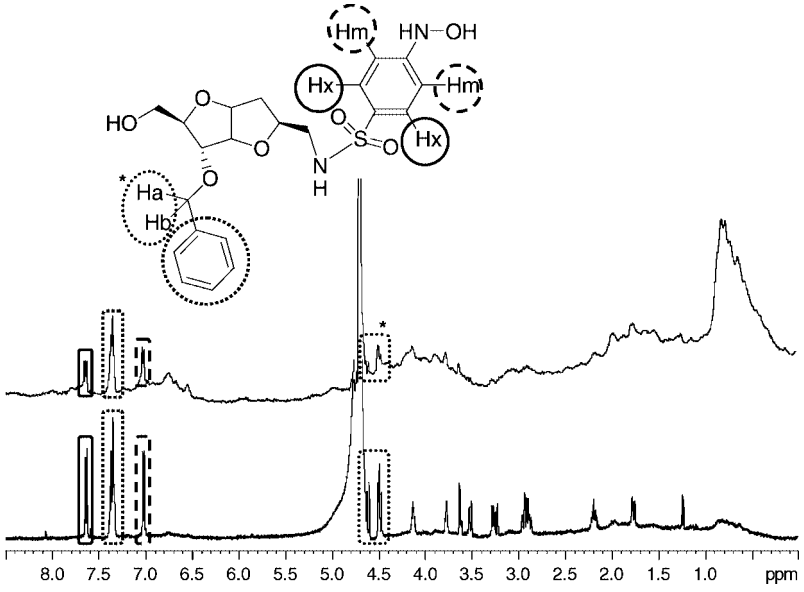


Figure 4. A) ^{1}H NMR of compound **5** with Ras-GDP complex; B) STD spectrum of compound **5** with Ras-GDP complex: ligand/protein ratio, 20:1, number of scan (NS) = 1360, on-resonance frequency δ = 0.8 ppm, off-resonance frequency δ = 40 ppm, total saturation time = 2 s. Spectra were recorded on the same sample, dissolved in a [D_{11}]-Tris buffer at pH = 7.3, containing 10% CD_3OD, 100 mM NaCl, and 5 mM $MgCl_2$. Total sample volume was 450 μL.

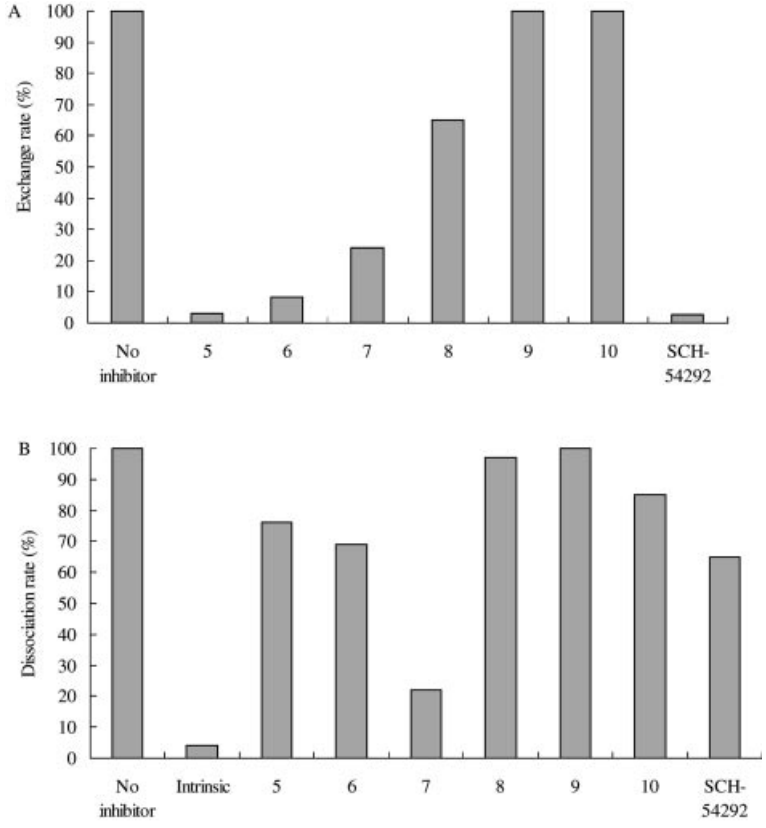


Figure 5. (A) C-Cdc25^{Mm}-stimulated nucleotide exchange on p21 hRas. The values are expressed as a percentage of the control exchange rate. (B) C-Cdc25^{Mm}-stimulated dissociation of p21 h-Ras·MANT-GDP complexes. The second column represents nucleotide dissociation due to Ras intrinsic activity. The values are expressed as a percentage of the control dissociation rate. The inhibitors (**5**–**10** and **SCH-54292**) were added at a final concentration of 100 μM.

Biology

In vitro Experiments on p21 h-Ras

Compounds **5**–**10** were initially tested for their ability to inhibit the C-Cdc25^{Mm}-stimulated nucleotide exchange on purified human Ras protein (p21 h-Ras). To this purpose, a modified version of Lenzen's method was used.[10] The C-Cdc25^{Mm}-stimulated guanine nucleotide exchange was monitored using the fluorescent 2′(3′)-*O*-(*N*-methylanthraniloyl)-GTP (MANT-GTP). p21 h-Ras was incubated with MANT-GTP in the absence and in the presence of 100 μM putative inhibitors **5**–**10**. **SCH-54292** was used as a positive control under the same experimental conditions. The exchange reaction was started by the addition of C-Cdc25^{Mm}. **SCH-54292**, **5** and **6** were the most active compounds, exhibiting 90% (**6**) or complete (**5** and **SCH-54292**) inhibition of the exchange. Lower activities were observed for **7** and **8**, (75% and 35% inhibition of nucleotide exchange, respectively), while compounds **9** and **10** were totally inactive (Figure 5A).

The mechanism of action of these inhibitors was also investigated in a nucleotide-dissociation assay (Figure 5B) by measuring the release of fluorescent nucleotide by the p21 h-Ras·MANT-GDP complex in the presence of GDP and exchange factor C-Cdc25^{Mm}.

The dissociation rate of p21 h-Ras in complex with MANT-GDP was partially reduced in the presence of compounds **SCH-54292**, **5** and **6**, and drastically reduced in the presence of compound **7**. Compounds **8**, **9** and **10** only slightly inhibited the GDP dissociation. Both the nucleotide exchange and dissociation experiments strongly suggest the importance of the inhibitor's benzyl group for the interaction with Ras: molecules **9** and **10**, which lack this group, did not show any interaction with the protein.

Effect of Ras Inhibitors on Mammalian Cells

In order to investigate a specific effect of Ras-mediated signalling in vivo, inhibition of mammalian cell growth by compounds **5**, **6**, **7** and **8** was evaluated both in normal cells and in cells transformed by k-Ras (Arg12).[11] Compounds **5** and **6** inhibited only slightly the growth of both cell lines when added at a concentration of 100 μM, while growth was completely blocked in both cell lines using compounds **7** and **8** at a same concentration (Figure 6).

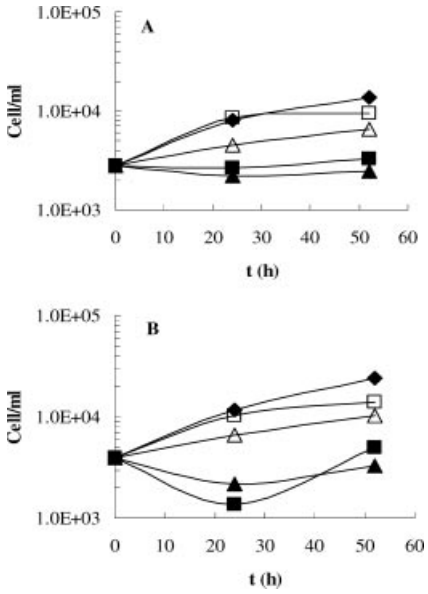


Figure 6. Inhibition test in mammalian cells. (A) Normal NIH3T3, and (B) NIH3T3 k-Ras mouse fibroblasts were seeded into 60 mm dishes and grown for 1 d: Three dishes were allowed to continue growing without addition of the inhibitor (♦), while compounds **5** (□), **6** (Δ), **7** (■) and **8** (▲) were added to the other dishes at a final concentration of 100 μM. At different time points sample cells were collected for determination of cell number.

In order to study the in vivo inhibition of the Ras-mediated signalling, preliminary experiments were performed to measure the level of activation of MAPK after addition of the inhibitors **5**, **6**, **7** and **8** to normal NIH3T3 fibroblasts growing in 10% serum. As shown in Figure 7, a pronounced decrease in phospho-MAPK expression was observed after 2 h at a final concentration of 100 μM for compound **7**, a less pronounced decrease was observed for compound **8**, while no difference in phospho-MAPK expression was observed for compounds **5** and **6** compared with the control. Since compounds **5** and **6** were the most potent inhibitors in vitro, the fact that they are less active than **7** and **8** in cell growth inhibition and completely inactive in decreasing the phospho-MAPK expression suggests that the polar sulfonamide group can prevent the crossing of the cell membrane.

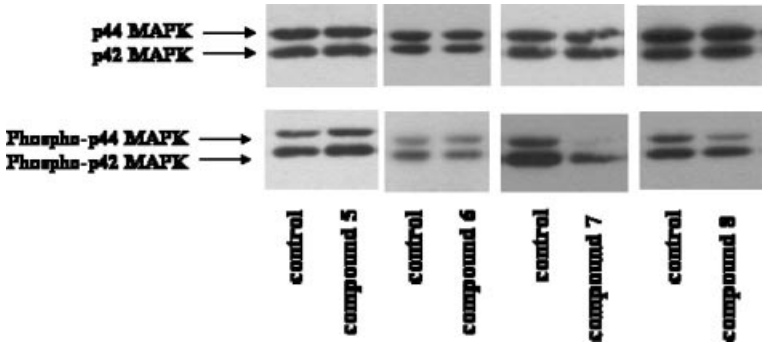


Figure 7. Assay of MAPK activation. Lysates (14 μg of total proteins) were separated by SDS-PAGE, transferred to nitrocellulose, and immunodecorated with anti-p42/44 MAPK antibody and anti-phospho-p42/44 MAPK antibody.

Conclusions

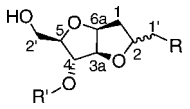
The data that we have presented here allow some general considerations on the structure-activity relationship of bicyclic Ras inhibitors. The epitope mapping through STD-NMR experiments for compounds **5**–**8** clearly points out that both the phenylhydroxylamino and the benzyl moieties of the ligands directly interact with Ras. On the contrary, the protons on the bicyclic scaffold do not interact directly with the protein. Both the benzyl and the phenylhydroxylamino groups can therefore be considered pharmacophores. The observation that compounds **9** and **10**, which lack both benzyl groups, are totally inactive in inhibiting nucleotide exchange and dissociation and Ras-dependent cellular proliferation, supports our hypothesis that the phenylhydroxylamino group must be accompanied by another aromatic moiety to have binding and biological activity.

The D-arabinose-derived bicyclic scaffold has the function of orienting the pharmacophore groups without directly interacting with the protein. STD-NMR experiments showed that the inhibitor orientation in the binding pocket of Ras was not strongly affected by the C-2 configuration and the nature (amide or sulfonamide) of the C-2 linker. However, these molecules have different behaviors in in vitro tests on p21 h-Ras: while **5** and **6** are more potent in blocking nucleotide exchange, compound **7** is the strongest inhibitor of the GDP dissociation. Moreover, only amide-containing molecules **7** and **8** are active in inhibiting cellular growth in both normal and k-Ras-transformed mammalian cells, while sulfonamides **5** and **6** are inactive. These data suggest that, while interacting more strongly with purified Ras, the sulfonamide moiety can prevent cellular uptake, thus decreasing their potency in cells. We are presently investigating the molecular mechanism of action of these inhibitors, with particular attention to their interactions with effectors and exchange factors.

Experimental Section

Chemistry

General Procedures: All solvents were dried with molecular sieves (4 Å, Fluka) for at least 24 h prior to use. When dry conditions were required, the reactions were performed under argon. Thin-layer chromatography (TLC) was performed on silica gel 60 F254 plates (Merck) with UV detection, or by using a developing solution of concd. H_2SO_4/EtOH/H_2O (5:45:45), followed by heating at 180 °C. Flash column chromatography was performed on silica gel 230–400 mesh (Merck). Mixtures of petroleum ether (boiling range 40–60 °C) and ethyl acetate were used as eluents. ^{1}H and ^{13}C NMR spectra were recorded with a Varian 400 MHz Mercury instrument at 300 K unless otherwise stated. Chemical shifts are reported in ppm downfield from TMS as internal standard; hydrogen numbering is shown in Scheme 3. Mass spectra were recorded with a Fourier Transform Ion Cyclotron Resonance (FT-ICR) instrument (model APEXII, Bruker Daltonics), equipped with a 4.7 T cryomagnet (Magnex). Optical rotations were measured at ambient temperature, using the sodium-D line, with a P3002 electronic polarimeter (A. Krüss, Germany).



Scheme 3. Hydrogen numbering in the bicyclic scaffold.

Methyl D-Arabinofuranoside (11): To a stirred suspension of D-arabinose (5 g, 33.304 mmol) in dry CH_3OH (200 mL), acetyl chloride was added dropwise at room temperature under argon. After 24 h, Amberlite IRN78 OH^- resin was added to neutralise the acid, and the mixture was stirred for 5 min. The resin was removed by filtration, and the solvent was evaporated. The product was purified by flash column chromatography (9.75:0.25, EtOAc/CH_3OH) affording **11** (4.96 g, 90% yield) as a yellow oil (mixture of α and β anomers).[12]

1-(D-Arabinofuranosyl)-2-propene (12): To a stirred solution of methyl D-arabinofuranoside (**11**) (4.5 g, 27.41 mmol) in dry CH_3CN (9 mL), BTSFA (16.3 mL, 61.670 mmol) was added at 100 °C under argon (balloon). After 3 h, the reaction mixture was cooled to room temperature. ATMS (6.5 mL, 41.115 mmol) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) (2.5 mL, 13.705 mmol) were added at 0 °C, and the reaction mixture was stirred at room temperature for 1 h. Water (120 mL) was added slowly to hydrolyze TMS ethers, the mixture was neutralised with 1 M NaOH, and concentrated in vacuo. The product was purified by flash column chromatography (1:9, petroleum ether/EtOAc) providing **12** (4.65 g, 97%) as a light green oil (mixture of α and β anomers; α/β = 1:1.5, as determined from the integration ratio of the ^{1}H NMR signals). HRMS (FT-ICR): $C_8H_{14}O_4Na$: calcd. 197.0790; found 197.0811 $[M+Na]^+$. The following signal attributions were obtained from the spectra of the mixture. **α Anomer:** ^{1}H NMR (CD_3OD): δ = 5.88 (tdd, J = 16.0, 6.9, 2.1 Hz, 1 H), 5.12 (ddd, J = 17.2, 3.6, 1.5 Hz, 1 H), 5.06 (m, 1 H), 3.95 (m, 1 H), 3.79 (m, 3 H), 3.68 (dd, J = 11.7, 3.4 Hz, 1 H), 3.60 (dd, J = 11.8, 5.4 Hz, 1 H), 2.39 (m, 2 H) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 135.5, 117.4, 84.7, 83.6, 81.6, 78.8, 63.3, 38.7 ppm. **β Anomer:** ^{1}H NMR (CD_3OD): δ = 5.87 (tdd, J = 17.2, 10.2, 7.0 Hz, 1 H), 5.13 (ddd, J = 17.2, 3.5, 1.5 Hz, 1 H), 5.03 (m, 1 H), 3.97 (m, 1 H), 3.95 (dd, J = 7.0, 3.2 Hz, 1 H), 3.82 (dd, J = 3.0 Hz, 1.0 Hz, 1 H), 3.75 (ddd, J = 4.8, 3.8, 2.6 Hz, 1 H), 3.67 (dd, J = 11.4, 3.8 Hz, 1 H), 3.64 (dd, J = 11.4, 4.9 Hz, 1 H), 2.40 (m, 2 H) ppm. ^{13}C NMR (CD_3OD): δ = 135.9, 117.1, 87.3, 82.5, 80.3, 78.5, 63.5, 34.3 ppm.

(2*R*,3a*S*,4*R*,5*R*,6a*S*)- and (2*S*,3a*S*,4*R*,5*R*,6a*S*)-4-Hydroxy-5-(hydroxymethyl)-2-(iodomethyl)hexahydrofuro[3,2-*b*]furan (*R*-13 and *S*-13): To a stirred solution of **12** (mixture of anomers, 4 g, 23 mmol) in dry THF (200 mL), NIS (7.7 g, 34.4 mmol) was added at 90 °C under argon. After 10 min, the β anomer had reacted completely according to TLC analysis (eluent: 3:7, petroleum ether/EtOAc), while the α anomer had not reacted, as expected.[3,4] The mixture was cooled to room temperature, $Na_2S_2O_3$ was added to reduce excess iodine, and the suspension was vigorously stirred until it became colourless. The product was purified by flash column chromatography (2:8, petroleum ether/EtOAc) to give **13** (4 g, 58%) as a light green oil [mixture of diastereomers, (*R*)/(*S*) = 2.5:1, as determined from the integration ratio of the ^{1}H NMR signals]. The α anomer was recovered unreacted. The following signal attributions were obtained from the spectra of the mixture. HRMS (FT-ICR): $C_8H_{13}IO_4Na$: calcd. 322.9756; found 322.9743 $[M+Na]^+$. $C_8H_{13}IO_4$ (300.1): calcd. C 32.02, H 4.37; found C 32.11, H 4.30. ***R*-13:** ^{1}H NMR (CD_3OD): δ = 4.73 (bt, J = 4.3 Hz, 1 H, 6a-H), 4.47 (dd, J = 4.2, 1.6 Hz, 1 H, 3a-H), 4.04 (m, 1 H, 2-H), 3.94 (dd, J = 5.3, 1.4 Hz, 1 H, 4-H), 3.70 (m, 2 H, 5-H, 2′a-H), 3.58 (dd, J

= 12.5, 7.1 Hz, 1 H, 2′b-H), 3.32 (m, 2 H, 1′a-H, 1′b-H), 2.28 (dd, *J* = 13.6, 5.3 Hz, 1 H, 1a-H), 1.67 (ddd, *J* = 13.7, 9.6, 4.6 Hz, 1 H, 1b-H) ppm. ^{13}C NMR (CD_3OD): δ = 92.6, 87.9, 84.3, 79.2, 78.6, 63.0, 40.5, 9.7 ppm. ***S*-13:** ^{1}H NMR (CD_3OD), selected signals: δ = 4.35 (dd, *J* = 4.3, 1.1 Hz, 1 H, H-3a), 4.20 (m, 1 H, H-2), 3.63 (dd, *J* = 12.5, 7.0 Hz, 1 H, 2′b-H), 2.03 (dd, *J* = 14.0, 4.4 Hz, 1 H, 1b-H) ppm. ^{13}C NMR (CD_3OD): δ = 93.7, 89.3, 84.5, 82.1, 78.5, 63.2, 38.8, 10.3 ppm.

(2*R*,3a*S*,4*R*,5*R*,6a*S*)- and (2*S*,3a*S*,4*R*,5*R*,6a*S*)-2-(Azidomethyl)-4-hydroxy-5-(hydroxymethyl)-hexahydrofuro[3,2-*b*]furan (*R*-14 and *S*-14): To a stirred solution of **13** (mixture of diastereomers, 2.2 g, 7.33 mmol) in dry DMF (35 mL) tetrabutylammonium azide[3] was added at 70 °C under argon. After 72 h, the reaction mixture was concentrated in vacuo without heating. The product was purified by flash column chromatography (2:8, petroleum ether/EtOAc) recovering **14** (1.41 g, 90%) as a yellow oil [mixture of diastereomers, (*R*)/(*S*) = 2.5:1, as determined from the integration ratio of the ^{1}H NMR signals]. HRMS (FT-ICR): $C_8H_{13}N_3O_4Na$: calcd. 238.0804; found 238.0789 $[M+Na]^+$. $C_8H_{13}N_3O_4$ (238.08): calcd. C 44.65, H 6.09, N 19.53; found C 44.59, H 6.12, N 19.49. ***R*-14:** ^{1}H NMR (CD_3OD): δ = 4.73 (bt, *J* = 4.4 Hz, 1 H, 6a-H), 4.44 (dd, *J* = 4.2, 1 H, 1.6 Hz, 3a-H), 4.28 (m, 1 H, 2-H), 3.97 (dd, *J* = 5.3, 1.4 Hz, 1 H, 4-H), 3.73–3.69 (m, 2 H, H-2′a, 5-H), 3.59 (dd, *J* = 12.5, 7.0 Hz, 1 H, 2′b-H), 3.48 (dd, *J* = 13.1, 3.3 Hz, 1 H, 1′a-H), 3.21 (dd, *J* = 13.1, 5.4 Hz, 1 H, 1′b-H) 2.10 (dd, *J* = 13.4, 5.4 Hz, 1 H, 1a-H), 1.78 (ddd, *J* = 13.4, 10.0, 4.7 Hz, 1 H, 1b-H) ppm. ^{13}C NMR (CD_3OD): δ = 92.3, 88.0, 84.2, 79.1, 78.6, 63.1, 54.7, 36.7 ppm. ***S*-14:** ^{1}H NMR (CD_3OD): δ = 4.69 (bt, *J* = 5.0 Hz, 1 H, 6a-H), 4.32 (d, *J* = 4.1 Hz, 1 H, 3a-H), 4.18 (m, 1 H, 2-H), 4.04 (d, *J* = 5.5 Hz, 1 H, 4-H), 3.73–3.69 (m, 2 H, 2′a-H, 5-H), 3.65 (dd, *J* = 11.8, 6.4 Hz, 1 H, 2′b-H), 3.49 (dd, *J* = 12.9, 4.0 Hz, 1 H, 1′a-H), 3.27 (dd, *J* = 12.9, 3.8 Hz, 1 H, 1′b-H), 2.25 (ddd, *J* = 14.1, 8.4, 6.0 Hz, 1 H, 1a-H) 1.88 (dd, *J* = 14.1, 5.3 Hz, 1 H, 1b-H) ppm. ^{13}C NMR (CD_3OD): δ = 93.4, 89.7, 84.3, 81.0, 78.3, 63.2, 55.8, 36.4 ppm.

(2*R*,3a*S*,4*R*,5*R*,6a*S*)- and (2*S*,3a*S*,4*R*,5*R*,6a*S*)-2-(Aminomethyl)-4-hydroxy-5-(hydroxymethyl)-hexahydrofuro[3,2-*b*]furan (*R*-15 and *S*-15): A solution of compound **14** (mixture of diastereomers, 500 mg, 2.35 mmol) in THF (20 mL) was treated with triphenylphosphane (1.86 g, 6.97 mmol) and H_2O (1.7 mL, 93 mmol), and was stirred at 60 °C overnight. The reaction mixture was concentrated in vacuo. The product was purified by flash column chromatography (8:2, EtOAc/CH_3OH, 1% TEA) affording **15** (377 mg, 85%) as a yellow oil [mixture of diastereomers, (*R*)/(*S*) = 2:1, as determined from the integration ratio of the ^{1}H NMR signals]. HRMS (FT-ICR): $C_8H_{15}NO_4Na$: calcd. 212.0899; found 212.0911 $[M+Na]^+$. $C_8H_{15}NO_4$ (212.08): calcd. C 50.78, H 7.99, N 7.40; found C 50.82, H 7.90, N 7.49. ***R*-15:** ^{1}H NMR (CD_3OD): δ = 4.71 (bt, *J* = 4.6 Hz, 1 H, 6a-H), 4.42 (dd, *J* = 4.3, 1.8 Hz, 1 H, 3a-H), 4.09 (m, 1 H, 2-H), 3.94 (dd, *J* = 5.8, 1.8 Hz, 1 H, 4-H), 3.88–3.57 (m, 3 H, 2′a-H, 2′b-Hb, 5-H), 2.81 (dd, *J* = 13.1, 3.9 Hz, 1 H, 1′a-H), 2.68 (dd, *J* = 13.1, 6.8 Hz, 1 H, 1′b-H), 2.09 (dd, *J* = 13.4, 5.1 Hz, 1 H, 1a-H), 1.61 (ddd, *J* = 13.5, 10.3, 4.8 Hz, 1 H, 1b-H) ppm. ^{13}C NMR (CD_3OD): δ = 91.9, 87.6, 84.1, 80.3, 78.6, 63.0, 45.8, 37.2 ppm. ***S*-15:** ^{1}H NMR (CD_3OD), selected signals: δ = 4.68 (bt, *J* = 4.4 Hz, 1 H, 6a-H), 4.28 (d, *J* = 4.3 Hz, 1 H, 3a-H), 2.85 (dd, *J* = 13.2, 6.2 Hz, 1 H, 1′a-H), 2.27 (ddd, *J* = 14.2, 8.4, 6.0 Hz, 1 H, 1a-H) 1.85 (dd, *J* = 14.4, 5.9 Hz, 1 H, 1b-H) ppm. ^{13}C NMR (CD_3OD): δ = 92.9, 89.8, 84.4, 81.7, 77.8, 63.0, 46.3, 36.3 ppm.

(2*R*,3a*S*,4*R*,5*R*,6a*S*)- and (2*S*,3a*S*,4*R*,5*R*,6a*S*)-2-(Azidomethyl)-4-benzyloxy-5-(benzyloxymethyl)-hexahydrofuro[3,2-*b*]furan (*R*-16 and *S*-16): To a stirred solution of compound **14** (mixture of diastereomers, 700 mg, 3.25 mmol) in dry DMF, benzyl bromide (1.55 mL, 13.018 mmol) and NaH (60% in oil, 520 mg, 13.01 mmol) were added in three portions at room temperature over a period of 20 min. After 15 min, the reaction was quenched by adding ethanol, and the solvents were evaporated. The product was purified by flash column chromatography (9.25:0.75, petroleum ether/EtOAc) obtaining **16** (1.25 g, 90.5%) as a yellow oil [mixture of diastereomers, (*R*)/(*S*) = 2:1 as determined from the integration ratio of the ^{1}H NMR signals]. HRMS (FT-ICR): $C_{22}H_{25}N_3O_4Na$: calcd. 418.1743; found 418.1735 $[M+Na]^+$. $C_{22}H_{25}N_3O_4$ (418.17): calcd. C 66.82, H 6.37, N, 10.63; found C 67.53, H 7.01, N 11.21. ***R*-16:** ^{1}H NMR ($CDCl_3$): δ = 7.4–7.2 (m, 10 H, aromatic protons), 4.79 (bt, *J* = 5.2 Hz, 1 H, 6a-H), 4.71 (m, 1 H, 3a-H), 4.7–4.5 ($2AB_q$, 4 H, benzyl), 4.29 (m, 1 H, 2-H), 4.03 (ddd, *J* = 6.2, 6.2, 3.8 Hz, 1 H, 5-H), 3.86 (dd, *J* = 6.2, 1.2 Hz, 1 H, 4-H), 3.61 (dd, *J* = 10.5, 3.8 Hz, 1 H, 2′a-H), 3.59 (dd, *J* = 10.5, 6.2 Hz, 1 H, 2′b-H), 3.52 (dd, *J* = 13.0, 3.5 Hz, 1 H, 1′a-H), 3.24 (dd, *J* = 13.0, 3.5 Hz, 1 H, 1′b-H), 2.19 (dd, *J* = 13.4, 5.2 Hz, 1 H, 1a-H), 1.78 (ddd, *J* = 13.4, 10.2, 6.3 Hz, 1 H, 1b-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 88.72, 85.60, 83.53, 83.35, 77.70, 73.41, 72.10, 70.18, 53.60, 35.82 ppm. ***S*-16:** ^{1}H NMR ($CDCl_3$): δ = 7.4–7.2 (m, 10 H, aromatic protons), 4.72 (bt, *J* = 4.4 Hz, 1 H, 6a-H), 4.65–4.45 (m, 5 H, 3a-H, benzyl), 4.24 (m, 1 H, 2-H), 4.03 (ddd, *J* = 6.0, 3.7 Hz, 1 H, 5-H), 3.94 (bd, *J* = 6.0 Hz, 1 H, 4-H), 3.65 (dd, *J* = 10.3, 3.0 Hz, 1 H, 2′a-H), 3.58 (dd, *J* = 10.3, 5.9 Hz, 1 H, 2′b-H), 3.48 (dd, *J* = 12.5, 8.1 Hz, 1 H, 1′a-H), 3.16 (dd, *J* = 12.5, 4.0 Hz, 1 H, 1′b-H), 2.20 (ddd, *J* = 14.1, 8.4, 5.6 Hz, 1 H, 1a-H) 1.91 (m, 1 H, 1b-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 90.00, 85.40, 85.03, 83.63, 80.23, 73.64, 72.57, 70.00, 55.28, 35.80 ppm.

(2*R*,3a*S*,4*R*,5*R*,6a*S*)- and (2*S*,3a*S*,4*R*,5*R*,6a*S*)-2-(Aminomethyl)-4-(benzyloxy)-5-methoxyhexahydrofuro[3,2-*b*]furan (*R*-17 and *S*-17): A solution of compound **16** (740 mg, 1.87 mmol) in 4:1 Ac_2O/TFA (16 mL) was stirred at room temperature for 90 min. The reaction was quenched by adding a mixture of ice and 1 M NaOH, and the product was extracted with AcOEt. After the usual workup, the crude mixture was recovered as a yellow oil. The crude mixture was dissolved in dry MeOH (30 mL) and metallic Na was added in a catalytic amount under argon. The solution was stirred at room temperature for 30 min. Amberlite IRA-120 H^+ resin was added, and the mixture was stirred for 10 min. Then the resin was removed by filtration, and the solvent was evaporated. After the usual workup and chromatography (5.5:4.5, petroleum ether/AcOEt), a mixture of (2*R*,3a*S*,4*R*,5*R*,6a*S*)- and (2*S*,3a*S*,4*R*,5*R*,6a*S*)-2-(azidomethyl)-4-(benzyloxy)-5-methoxyhexahydrofuro[3,2-*b*]furan[4] [(*R*)/(*S*) = 2:1, as determined from the integration ratio of the ^{1}H NMR signals] was provided as a colourless oil (463 mg, 84.5% for the two steps). To a solution of this diastereomeric mixture (450 mg, 1.473 mmol) in THF (15 mL), triphenylphosphane (1.18 g, 4.420 mmol) and water (1 mL, 59 mmol) were added, and the reaction mixture was stirred at 60 °C overnight. The reaction mixture was concentrated in vacuo. The product was purified by flash column chromatography (7.5.2.5, EtOAc/MeOH with 1% TEA → 1:1, EtOAc/MeOH with 1% TEA), affording **17** (377 mg, 94%) as colourless oil [mixture of diastereomers, (*R*)/(*S*) = 2:1, as determined from the integration ratio of the ^{1}H NMR signals]. HRMS (FT-ICR): $C_{15}H_{21}NO_4Na$: calcd. 302.1368; found 302.1354 $[M+Na]^+$. $C_{15}H_{21}NO_4$ (302.13): calcd. C 64.50, H 7.58, N 5.01; found C 64.45, H 7.53, N 5.08. ***R*-17:** ^{1}H NMR (CD_3OD): δ = 7.34–7.32 (m, 5 H, aromatic protons), 4.71 (t, *J* = 4.5 Hz, 1 H, 6a-H), 4.68, 4.55 (ABq, *J* = 11.7 Hz, 2 H, benzyl), 4.59 (dd, *J* = 1.1, 4.3 Hz, 1 H, 3a-H), 4.09 (m, 1 H, 2-H), 3.90–3.79 (m, 2 H, 4-H, 5-H), 3.69 (dd, *J* = 11.8, 3.2 Hz, 1 H, 2′a-H), 3.58 (dd, *J* = 11.7,

5.4 Hz, 1 H, 2′b-H), 2.78 (dd, *J* = 13.2, 3.9 Hz, 1 H, 1′a-H), 2.66 (dd, *J* = 13.2, 6.6 Hz, 1 H, 1′b-H), 2.08 (dd, *J* = 13.4, 5.0 Hz, 1 H, 1a-H), 1.61 (ddd, *J* = 13.5, 10.3, 4.7 Hz, 1 H, 1b-H) ppm. ^{13}C NMR (CD_3OD): δ = 139.1, 129.2, 128.8, 128.6, 128.6, 89.6, 86.3, 86.0, 84.6, 80.9, 72.89, 63.1, 45.9, 37.1 ppm. ***S*-17:** ^{1}H NMR (CD_3OD), selected signals: δ = 4.44 (d, *J* = 4.0 Hz, 1 H, 3a-H), 2.24 (ddd, *J* = 14.0, 8.3, 6.1 Hz, 1 H, 1a-H), 1.83 (dd, *J* = 13.9, 4.9 Hz, 1 H, 1b-H) ppm. ^{13}C NMR (CD_3OD) selected signals: δ = 139.06, 90.1, 87.9, 85.6, 84.9, 82.7, 72.94, 63.2, 46.7, 36.3 ppm.

(2*R*,3a*S*,4*R*,5*R*,6a*S*)- and (2*S*,3a*S*,4*R*,5*R*,6a*S*)-4-Hydroxy-5-methoxy-2-[(4-nitrophenyl)sulfonamidomethyl]hexahydrofuro[3,2-*b*]furan (18): To a stirred solution of compound **15** (180 mg, 0.951 mmol) in dry pyridine (13.5 mL), *p*-nitrobenzensulfonyl chloride was added at 0 °C under argon. After 6 h, the reaction was quenched by adding CH_3OH. The solvents were evaporated in vacuo and, after flash chromatography (1:9, petroleum ether/EtOAc), **18** (228 mg, 64%) was recovered as pale yellow oil [diastereomeric mixture, (*R*)/(*S*) = 3:1, as determined from the integration ratio of the ^{1}H NMR signals). HRMS (FT-ICR): $C_{14}H_{18}N_2O_8SNa$: calcd. 397.0682; found 397.0674 $[M + Na]^+$. $C_{14}H_{18}N_2O_8S$ (374.2): C 44.92, H 4.85, N 7.48; found C 44.9, H 4.89, N 7.39. ***R*-18:** ^{1}H NMR (CD_3OD): δ = 8.39, 8.07 (AA′XX′, *J* = 8.8 Hz, 4 H, aromatic protons), 4.65 (bt, *J* = 4.5 Hz, 1 H, 6a-H), 4.28 (dd, *J* = 4.3, 1.8 Hz, 1 H, 3a-H), 4.06 (m, 1 H, 2-H), 3.81 (dd, *J* = 5.5, 1.5 Hz, 1 H, 4-H), 3.68–3.61 (m, 2 H, 2′a-H, 5-H), 3.50 (dd, *J* = 12.5, 7.1 Hz, 1 H, 2′b-H), 3.15 (dd, *J* = 13.6, 3.9 Hz, 1 H, 1′a-H), 3.02 (dd, *J* = 13.6, 5.8 Hz, 1 H, 1′b-H), 2.04 (dd, *J* = 13.5, 5.2 Hz, 1 H, 1a-H), 1.65 (ddd, *J* = 13.5, 10.1, 4.8 Hz, 1 H, 1b-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 151.1, 147.9, 129.2, 125.2, 92.1, 87.8, 84.0, 78.6, 78.5, 63.0, 47.2, 37.0 ppm. ***S*-18:** ^{1}H NMR (CD_3OD) selected signals: δ = 4.62 (bt, *J* = 4.6 Hz, 1 H, 6a-H), 4.22 (d, *J* = 4.0 Hz, 1 H, 3a-H), 3.95 (d, *J* = 5.3 Hz, 1 H, 4-H), 3.54 (dd, *J* = 11.7, 5.8 Hz, 1 H, 2′b-H), 2.18 (ddd, *J* = 14.1, 8.4, 5.8 Hz, 1 H, 1a-H), 1.84 (dd, *J* = 14.1, 4.8 Hz, 1 H, 1b-H) ppm. ^{13}C NMR ($CDCl_3$), selected signals: δ = 129.2, 93.2, 89.7, 84.4, 80.4, 78.1, 63.2, 36.3 ppm.

(2*R*,3a*S*,4*R*,5*R*,6a*S*)- and (2*S*,3a*S*,4*R*,5*R*,6a*S*)-4-Hydroxy-5-methoxy-2-[(4-nitrophenyl)carboxamidomethyl]hexahydrofuro[3,2-*b*]furan (19): To a stirred solution of compound **15** (200 mg, 1.057 mmol) in dry DMF (16 mL), HOBt (214 mg, 1.585 mmol), DIPEA (545 μL, 3.171 mmol) and *p*-nitrobenzoic acid (212 mg, 1.268 mmol) were added under argon. DIC (246 μL, 1.585 mmol) was added at 0 °C, and the reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated in vacuo, and the product was purified by flash column chromatography (8.5:1.5, toluene/CH_3OH) affording **19** (270 mg, 75.5%) as a pale yellow oil [mixture of diastereomers, (*R*)/(*S*) = 2:1, as determined from the integration ratio of the ^{1}H NMR signals]. HRMS (FT-ICR): $C_{15}H_{18}N_2O_7Na$: calcd. 361.1012; found 361.1033 $[M + Na]^+$. $C_{15}H_{18}N_2O_7$ (338.6): calcd. C 53.25, H 5.36, N 8.28; found C 53.19, H 5.29, N 8.36. ***R*-19:** ^{1}H NMR (CD_3OD): δ = 8.27, 7.99 (AA′XX′, *J* = 8.9 Hz, 4 H, aromatic protons), 4.72 (bt, *J* = 4.7 Hz, 1 H, 6a-H), 4.47 (dd, *J* = 4.4, 1.9 Hz, 1 H, 3a-H), 4.29 (m, 1 H, 2-H), 3.96 (dd, *J* = 5.8, 1.8 Hz, 1 H, 4-H), 3.74–3.65 (m, 3 H), 3.62–3.47 (m, 2 H, 1′a-H, 1′b-H), 2.17 (dd, *J* = 13.4, 5.2 Hz, 1 H, 1a-H), 1.68 (ddd, *J* = 13.5, 10.1, 4.8 Hz, 1 H, 1b-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 167.8, 150.6, 140.9, 129.4, 124.3, 92.0, 87.6, 83.9, 78.5, 78.4, 62.9, 44.4, 37.6 ppm. ***S*-19:** ^{1}H NMR (CD_3OD), selected signals: δ = 4.69 (bt, *J* = 5.2 Hz, 1 H, 6a-H), 4.12 (d, *J* = 5.1 Hz, 1 H, 3a-H), 2.28 (ddd, *J* = 14.1, 8.5, 5.8 Hz, 1 H, 1a-H), 1.95 (dd, *J* = 14.1, 5.2 Hz, 1 H, 1b-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 168.1, 150.5, 141.2, 129.6, 124.2, 93.0, 89.7, 84.5, 80.0, 77.9, 62.9, 45.2, 36.0 ppm.

(2*S*,3a*S*,4*R*,5*R*,6a*S*)- and (2*R*,3a*S*,4*R*,5*R*,6a*S*)-4-(Benzyloxy)-5-methoxy-2-[(4-nitrophenyl)sulfonamidomethyl]hexahydrofuro[3,2-*b*]-furan (20 and 21): To a stirred solution of compound **17** (15 mg, 0.053 mmol) in dry CH_2Cl_2 (0.7 mL), TEA (9 μL, 0.064 mmol) and *p*-nitrobenzensulfonyl chloride were added at 0 °C under argon. After 5 h, the reaction was quenched by adding MeOH. The solvents were evaporated in vacuo and, after flash chromatography (1:1, petroleum ether/EtOAc), pure compounds **20** [(*S*) diastereomer, 5 mg, 20%] and **21** [(*R*) diastereomer, 15 mg, 60%] were purified and separated as pale yellow oils. **20:** ^{1}H NMR ($CDCl_3$): δ = 8.31, 8.04 (AA′XX′, *J* = 8.8 Hz, 4 H, aromatic protons), 7.30–7.22 (m, 5 H, aromatic protons), 6.38 (dd, *J* = 6.8, 2.8 Hz, 1 H, NH), 4.65, 4.52 (ABq, *J* = 11.8 Hz, 2 H, benzyl), 4.65 (dd, *J* = 5.5, 3.5 Hz, 1 H, 6a-H), 4.40 (d, *J* = 3.4 Hz, 1 H, 3a-H), 4.31–4.26 (m, 1 H, 2-H), 4.07 (d, *J* = 5.4 Hz, 1 H, 4-H), 4.00–3.94 (m, 2 H, 2′a-H, 5-H), 3.72 (dd, *J* = 11.6, 2.0 Hz, 1 H, 2′b-H), 3.18 (dt, *J* = 12.6, 3.0 Hz, 1 H, 1′a-H), 3.10 (ddd, *J* = 12.6, 6.9, 3.4 Hz, 1 H, 1′b-H), 2.63 (br. s, 1 H, O*H*), 2.24 (ddd, *J* = 14.6, 9.4, 5.6 Hz, 1 H, 1a-H), 1.99 (dd, *J* = 14.6, 4.6 Hz, 1 H, 1b-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 149.65, 145.6, 137.1, 128.4, 128.2, 127.9, 127.5, 124.1, 89.2, 86.5, 83.5, 83.0, 77.9, 72.4, 61.3, 46.0, 33.9 ppm. $[\alpha]_D^{20}$ = +14.6 (*c* = 0.5, $CHCl_3$). HRMS (FT-ICR): $C_{21}H_{24}N_2O_8S$ Na: calcd. 487.1151; found 487.1143 $[M + Na]^+$. $C_{21}H_{24}N_2O_8S$ (464.2): calcd. C 54.30, H 5.21, N 6.03; found: C 54.22, H 5.29, N 5.97. **21:** ^{1}H NMR ($CDCl_3$): δ = 8.32, 8.03 (AA′XX′, *J* = 8.6 Hz, 4 H, aromatic protons), 7.37–7.29 (m, 5 H, aromatic protons) 5.33 (t, *J* = 6.1 Hz, 1 H, NH), 4.72 (bt, *J* = 4.5 Hz, 1 H, 6a-H), 4.63, 4.50 (ABq, *J* = 11.6 Hz, 2 H, benzyl), 4.55 (dd, *J* = 4.4, 1.5 Hz, 1 H, 3a-H), 4.12 (m, 1 H, 2-H), 3.86–3.76 (m, 3 H, 2′a-H, 4-H, 5-H), 3.60 (dd, *J* = 12.0, 4.8 Hz, 1 H, 2′b-H), 3.27 (ddd, *J* = 13.0, 6.2, 3.3 Hz, 1 H, 1′a-H), 3.03 (td, *J* = 12.9, 3.0 Hz, 1 H, 1′b-H), 2.10 (dd, *J* = 13.5, 5.0 Hz, 1 H, 1a-H), 2.05 (br. s, 1 H, OH), 1.68 (ddd, *J* = 13.5, 10.3, 4.7 Hz, 1 H, 1b-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 149.8, 145.5, 137.1, 128.4, 128.0, 127.8, 127.5, 124.3, 88.8, 84.4, 84.1, 83.0, 76.8, 72.2, 62.1, 45.9, 35.7 ppm. $[\alpha]_D^{20}$: +7.8 (c 0.5, $CHCl_3$). HRMS (FT-ICR): $C_{21}H_{24}N_2O_8S$ Na: calcd. 487.1151; found 487.1164 $[M + Na]^+$. $C_{21}H_{24}N_2O_8S$ (487.11): calcd. C 54.30, H 5.21, N 6.03; found C 54.26, H 5.25, N 6.00.

(2*S*,3a*S*,4*R*,5*R*,6a*S*)- and (2*R*,3a*S*,4*R*,5*R*,6a*S*)-4-(Benzyloxy)-5-methoxy-2-[(4-nitrophenyl)carboxamidomethyl]hexahydrofuro[3,2-*b*]furan (22 and 23): To a stirred solution of compound **17** (200 mg, 0.716 mmol) in dry DMF (10 mL), HOBt (145 mg, 1.074 mmol), DIPEA (368 μL, 2.148 mmol) and 4-nitrobenzoic acid (144 mg, 0.86 mmol) were added under argon. DIC (166 μL, 1.074 mmol) was added at 0 °C, and the reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated in vacuo and, after flash chromatography (4.5:5.5, toluene/EtOAc), pure compounds **22** [(*S*) diastereomer, 59 mg, 20%] and **23** [(*R*) diastereomer, 150 mg, 50%] were purified and separated as pale yellow oils. **22:** ^{1}H NMR ($CDCl_3$): δ = 8.23, 8.01 (AA′XX′, *J* = 8.9 Hz, 4 H, aromatic protons), 7.65 (dd, *J* = 6.8, 2.8 Hz, 1 H, NH), 7.35–7.28 (m, 5 H, aromatic protons), 4.69 (dd, *J* = 5.0, 3.3 Hz, 1 H, 6a-H), 4.66, 4.53 (ABq, *J* = 11.7 Hz, 2 H, benzyl), 4.41 (m, 2 H, 2-H, 3a-H), 4.09 (d, *J* = 5.0 Hz, 1 H, 4-H), 4.02 (m, 1 H, 5-H), 3.80 (m, 2 H, 1′a-H, 2′a-H), 3.69 (dd, *J* = 11.9, 2.9 Hz, 1 H, 2′b-H), 3.53 (td, *J* = 14.3, 3.0 Hz, 1 H, 1′b-H), 2.82 (br. s, 1 H, OH), 2.27 (ddd, *J* = 14.4, 9.0, 5.6 Hz, 1 H, 1a-H), 2.07 (dd, *J* = 14.4, 4.8 Hz, 1 H, 1b-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 166.3, 149.1, 140.2, 137.2, 128.514, 128.35, 127.8, 127.5, 123.2, 88.5, 86.8, 83.8, 83.2, 78.6, 72.3, 61.6, 43.4, 33.7 ppm. $[\alpha]_D^{20}$ = –18.4 (*c* = 0.5, $CHCl_3$). HRMS (FT-ICR): $C_{22}H_{24}N_2O_7Na$: calcd. 451.1481; found 451.1498 $[M + Na]^+$. $C_{22}H_{24}N_2O_7$ (428.4): calcd. C 61.67, H 5.65, N 6.54; found C 61.64, H 5.61, N 6.59. **23:** ^{1}H NMR ($CDCl_3$): δ = 8.26,

7.94 (AA′XX′, J = 8.7 Hz, 4 H, aromatic protons), 7.35–7.28 (m, 5 H, aromatic protons), 6.77 (t, J = 4.9 Hz, 1 H, NH) 4.75 (bt, J = 4.5 Hz, 1 H, 6a-H) 4.68, 4.54 (ABq, J = 11.6 Hz, 2 H, benzyl) 4.63 (d, J = 4.4 Hz, 1 H, 3a-H), 4.28 (m, 1 H, 2-H), 3.89 (m, 2 H, 4-H, 5-H), 3.82 (dd, J = 12.0, 2.2 Hz, 1 H, 2′a-H), 3.80 (m, 1 H, 1′a-H), 3.65 (dd, J = 12.0, 4.0 Hz, 1 H, 2′b-H), 3.49 (td, J = 13.8, 5.9 Hz, 1 H, 1′b-H), 2.50 (br. s, 1 H, OH), 2.19 (dd, J = 13.5, 4.9 Hz, 1 H, 1a-H), 1.64 (ddd, J = 13.6, 10.5, 4.7 Hz, 1 H, 1b-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 165.3, 149.5, 139.6, 137.2, 128.3, 128.1, 127.8, 127.5, 123.6, 88.8, 84.5, 84.2, 83.1, 77.4, 72.1, 62.2, 43.0, 36.1 ppm. $[\alpha]_D^{20}$ = –17.2 (c = 0.5, $CHCl_3$). HRMS (FT-ICR): $C_{22}H_{24}N_2O_7Na$: calcd. 451.1481; found 451.1476 $[M + Na]^+$. $C_{22}H_24N_2O_7$ (428.2): calcd. C 61.67, H 5.65, N 6.54; found C 61.69, H 5.60, N 6.57.

(2*S*,3a*S*,4*R*,5*R*,6a*S*)-4-(Benzyloxy)-2-{[4-(hydroxyamino)phenyl]-sulfonamidomethyl}-5-methoxyhexahydrofuro[3,2-*b*]furan (5): To a stirred solution of compound **20** (40 mg, 0.086 mmol) in THF (3.7 mL), Pd/C (3.7 mg) was added at 0 °C. After 15 min, the suspension was treated with hydrazine hydrate (8.4 μL, 0.172 mmol) and stirred at 0 °C for 45 min. The reaction was quenched by adding acetone. The Pd/C was removed by filtration, and the solvents were concentrated in vacuo. The product was purified by flash column chromatography (9.5:0.5 toluene/CH_3OH) to give **5** (35.5 mg, 92%) as a pale yellow amorphous solid. ^{1}H NMR (9:1, D_2O/CD_3OD): δ = 7.62, 7.00 (AA′XX′, J = 8.8 Hz, 4 H, aromatic protons), 7.38–7.32 (m, 5 H, aromatic protons), 4.64 (bt, J = 5.0 Hz, 1 H, 6a-H), 4.59, 4.47 (ABq, J = 11.6 Hz, 2 H, benzyl), 4.48 (bd, J = 4.7 Hz, 1 H, 3a-H), 4.11 (m, 1 H, 2-H), 3.76 (m, 1 H, 5-H) 3.61 (bd, J = 6.1 Hz, 1 H, 4-H), 3.49 (dd, J = 12.2, 3.4 Hz, 1 H, 2′a-H), 3.26 (dd, J = 12.2, 6.6 Hz, 1 H, 2′b-H), 2.93 (dd, J = 13.3, 3.9 Hz, 1 H, 1′a-H), 2.87 (dd, J = 13.3, 8.0 Hz, 1 H, 1′b-H), 2.18 (ddd, J = 14.4, 8.7, 5.9 Hz, 1 H, 1a-H), 1.75 (dd, J = 14.6, 4.0 Hz, 1 H, 1b-H) ppm. ^{13}C NMR δ = 156.7, 139.0, 131.05, 129.2, 129.1, 128.8, 128.6, 113.1, 90.4, 87.7, 85.9, 84.7, 80.6, 72.9, 63.4, 48.8, 36.3 ppm. $[\alpha]_D^{20}$ = –12.65 (c = 0.7, DMSO). HRMS (FT-ICR): $C_{21}H_{26}N_2O_7SNa$: calcd. 473.1358; found 473.1367 $[M + Na]^+$. $C_{21}H_{26}N_2O_7S$ (450.6): calcd. C 55.99, H 5.82, N 6.22; found C 56.07, H 5.77, N 6.29.

(2*R*,3a*S*,4*R*,5*R*,6a*S*)-4-(Benzyloxy)-2-{[4-(hydroxyamino)phenyl]-sulfonamidomethyl}-5-methoxyhexahydrofuro[3,2-*b*]furan (6): Compound **21** (50 mg, 0.107 mmol) was allowed to react under the conditions described for compound **5** to afford compound **6** (45 mg, 92%) as a pale yellow amorphous solid. ^{1}H NMR (9:1, D_2O/CD_3OD): δ = 7.68, 7.02 (AA′XX′, J = 8.1 Hz, 4 H, aromatic protons), 7.39–7.34 (m, 5 H, aromatic protons), 4.67 (bt, J = 5.0 Hz, 1 H, 6a-H), 4.60, 4.48 (ABq, J = 11.5 Hz, 2 H, benzyl), 4.42 (bd, J = 4.7 Hz, 1 H, 3a-H), 3.95 (m, 1 H, 2-H), 3.75 (m, 1 H, 5-H) 3.65 (bd, J = 5.7 Hz, 1 H, 4-H), 3.59 (dd, J = 12.5, 3.4 Hz, 1 H, 2′a-H), 3.42 (dd, J = 12.5, 5.8 Hz, 1 H, 2′b-H), 3.14 (dd, J = 13.7, 3.9 Hz, 1 H, 1′a-H), 2.97 (dd, J = 13.7, 5.3 Hz, 1 H, 1′b-H), 1.95 (dd, J = 14.3, 3.6 Hz, 1 H, 1a-H), 1.61 (ddd, J = 14.3, 8.7, 5.9 Hz, 1 H, 1b-H) ppm. ^{13}C NMR (CD_3OD): δ = 155.6, 138.6, 129.9, 128.8, 128.4, 128.2, 128.1, 112.1, 88.4, 85.7, 85.6, 83.2, 77.9, 71.6, 62.2, 46.7, 36.9 ppm. $[\alpha]_D^{20}$: –4.75 (c 0.5, DMSO). HRMS (FT-ICR): $C_{21}H_{26}N_2O_7SNa$: calcd. 473.1358; found 473.1342 $[M + Na]^+$. $C_{21}H_{26}N_2O_7S$ (450.6): calcd. C 55.99, H 5.82, N 6.22; found C 56.04, H 5.76, N 6.25.

(2*S*,3a*S*,4*R*,5*R*,6a*S*)-4-(Benzyloxy)-2-{[4-(hydroxyamino)phenyl]-carboxamidomethyl}-5-methoxyhexahydrofuro[3,2-*b*]furan (7): Compound **22** (70 mg, 0.163 mmol) was allowed to react under the conditions described for compound **5** and, after purification by flash column chromatography (9:1, toluene/MeOH), afforded **7** (62 mg, 92%) as a pale yellow amorphous solid. ^{1}H NMR (9:1, D_2O/CD_3OD): δ = 7.60, 6.99 (AA′XX′, J = 8.7 Hz, 4 H, aromatic protons), 7.28 (m, 5 H, aromatic protons), 4.72 (m, 1 H, 6a-H), 4.61, 4.48 (ABq, J = 11.6 Hz, 2 H, benzyl), 4.52 (bd, J = 4.2 Hz, 1 H, 3a-H), 4.27 (m, 1 H, 2-H), 3.84 (m, 2 H, 4-H, 5-H), 3.61 (dd, J = 12.3, 3.6 Hz, 1 H, 2′a-H), 3.54 (dd, J = 13.9, 7.1 Hz, 1 H, 1′a-H), 3.51 (m, 1 H, 2′b-H), 3.38 (dd, J = 14.0, 3.8 Hz, 1 H, 1′b-H), 2.27 (ddd, J = 14.5, 8.0, 6.3 Hz, 1 H, 1a-H), 1.86 (dd, J = 14.6, 4.0 Hz, 1 H, 1b-H) ppm. ^{13}C NMR (CD_3OD): δ = 170.1, 156.0, 139.1, 129.3, 129.2, 128.8, 128.6, 126.4, 113.3, 90.4, 87.9, 85.8, 84.9, 80.7, 73.0, 63.3, 45.3 36.3 ppm. $[\alpha]_D^{20}$ = –3.91 (c = 0.7, DMSO). HRMS (FT-ICR): $C_{22}H_{26}N_2O_6Na$: calcd. 437.1689; found 437.1678 $[M + Na]^+$.$C_{22}H_{26}N_2O_6$ (414.3): calcd. C 63.76, H 6.32, N 6.76; found C 63.73, H 6.29, N 6.81.

(2*R*,3a*S*,4*R*,5*R*,6a*S*)-4-(Benzyloxy)-2-{[4-(hydroxyamino)phenyl]-carboxamidomethyl}-5-methoxyhexahydrofuro[3,2-*b*]furan (8): The procedure was carried out as described for the preparation of **5**, starting from **23** (90 mg, 0.209 mmol) and, after purification by flash column chromatography (9:1, toluene/CH_3OH), afforded **8** (82 mg, 95%) as pale yellow amorphous solid. ^{1}H NMR (9:1, D_2O/CD_3OD): δ = 7.65, 7.02 (AA′XX′, J = 8.8 Hz, 4 H, aromatic protons), 7.32–7.26 (m, 5 H, aromatic protons), 4.74 (m, 1 H, 6a-H), 4.63, 4.53 (ABq, J = 11.6 Hz, 2 H, benzyl), 4.62 (m, 1 H, 3a-H), 4.23 (m, 1 H, 2-H), 3.82 (dd, J = 6.4, 1.4 Hz, 1 H, 4-H), 3.79 (m, 1 H, 5-H), 3.65 (dd, J = 12.3, 3.1 Hz, 1 H, 2′a-H), 3.51 (dd, J = 13.3, 5.8 Hz, 1 H, 2′b-H), 3.48 (m, 1 H, 1′a-H), 3.43 (dd, J = 14.2, 6.4 Hz, 1 H, 1′b-H), 2.11 (dd, J = 13.9, 5.3 Hz, 1 H, 1a-H), 1.68 (ddd, J = 14.2 10.2, 4.9 Hz, 1 H, 1b-H) ppm. ^{13}C NMR (CD_3OD): δ = 170.0, 156.1, 139.0, 129.2, 129.2, 128.8, 128.6, 126.3, 113.3, 89.7, 86.0, 86.0, 84.5, 79.1, 72.9, 63.1, 44.1, 37.6 ppm. $[\alpha]_D^{20}$ = –13.59 (c = 0.7, DMSO). HRMS (FT-ICR): $C_{22}H_{26}N_2O_6Na$: calcd. 437.1689; found 437.1698 $[M + Na]^+$. $C_{22}H_{26}N_2O_6$ (414.3): calcd. C 63.76, H 6.32, N 6.76; found C 63.75, H 6.28, N 6.83.

(2*R*,3a*S*,4*R*,5*R*,6a*S*)- and (2*S*,3a*S*,4*R*,5*R*,6a*S*)-4-Hydroxy-2-{[4-(hydroxyamino)phenyl]sulfonamidomethyl}-5-methoxyhexahydrofuro-[3,2-*b*]furan (*R*-9 and *S*-9): The procedure was carried out as described for the preparation of **5**, starting from **18** (100 mg, 0.267 mmol) and, after purification by flash column chromatography (7.5:2.5, toluene/MeOH), afforded **9** (95 mg, 98%) as pale yellow amorphous solid [mixture of diastereomers, (*R*)/(*S*) = 3:1, as determined from the integration ratio of the ^{1}H NMR signals]. ***R*-9:** ^{1}H NMR (D_2O): δ = 7.70, 7.09 (AA′XX′, J = 8.8 Hz, 4 H, aromatic protons), 4.70 (m, 1 H, 6a-H), 4.29 (dd, J = 4.3, 1.8 Hz, 1 H, 3a-H), 4.02 (m, 1 H, 2-H), 3.82 (dd, J = 5.8, 1.7 Hz, 1 H, 4-H), 3.71 (ddd, J = 6.4, 6.2, 3.5 Hz, 1 H, 5-H), 3.65 (dd, J = 12.3, 3.5, 3.5 Hz, 1 H, 2′a-H), 3.49 (dd, J = 12.2, 6.6 Hz, 1 H, 2′b-H), 3.11 (dd, J = 14.1, 3.6 Hz, 1 H, 1′a-H), 3.02 (dd, J = 13.6, 5.8 Hz, 1 H, 1′b-H), 1.98 (dd, J = 14.0, 5.4 Hz, 1 H, 1a-H), 1.64 (ddd, J = 14.3, 10.2, 4.8 Hz, 1 H, 1b-H) ppm. ^{13}C NMR (CD_3OD): δ = 156.6, 131.3, 129.1, 113.1, 92.1, 87.7, 84.1, 78.6, 78.5, 63.2, 47.2, 37.2 ppm. ***S*-9:** ^{1}H NMR (D_2O), selected signals: δ = 4.33 (dd, J = 4.2, 0.8 Hz, 1 H, H-3a), 4.11(m, 1 H, H-2) 3.85 (dd, J = 5.5, 0.7 Hz, 1 H, 4-H), 3.75 (m, 1 H, 5-H), 3.60 (dd, J = 12.1, 3.7 Hz, 1 H, 2′a-H), 3.42 (dd, J = 12.1, 6.7 Hz, 1 H, 2′b-H), 2.21 (ddd, J = 14.3, 8.5, 5.8 Hz, 1 H, 1a-H), 1.75 (dd, J = 14.6, 4.6 Hz, 1 H, H-1b) ppm. ^{13}C NMR (CD_3OD) selected signals: δ = 131.1, 129.2, 113.0, 93.1, 89.6, 84.4, 80.4, 78.3, 63.5, 36.5 ppm. HRMS (FT-ICR): $C_{14}H_{20}N_2O_7SNa$: calcd. 383.0889; found 383.0877 $[M + Na]^+$. $C_{14}H_{20}N_2O_7S$ (360.2): calcd. C 46.66, H 5.59, N 7.77; found C 46.59, H 5.63, N 7.81.

(2*R*,3a*S*,4*R*,5*R*,6a*S*)- and (2*S*,3a*S*,4*R*,5*R*,6a*S*)-2-{[4-(Hydroxyamino)phenyl]carboxamidomethyl}-5-methoxyhexahydrofuro-[3,2-*b*]furan (*R*-10 and *S*-10): The procedure was carried out as described for the preparation of **5**, starting from **19** (120 mg, 0.355 mmol) and, after purification by flash column chromatography (7.5:2.5, toluene/CH_3OH), pure **10** (113 mg, 98%) was obtained as a pale yellow amorphous solid [mixture of diastereomers, (*R*)/(*S*) = 2:1, as determined from the integration ratio of the ^1H NMR signals]. HRMS (FT-ICR): $C_{15}H_{20}N_2O_6Na$: calcd. 347.1219; found 347.1234 $[M+Na]^+$. $C_{15}H_{20}N_2O_6$ (324.3): calcd. C 55.55, H 6.22, N 8.64; found C 55.63, H 6.29, N 8.57. ***R*-10:** ^1H NMR (D_2O): δ = 7.65, 7.02 (AA′XX′, *J* = 8.8 Hz, 4 H, aromatic protons), 4.77 (bt, *J* = 4.6 Hz, 1 H, 6a-H), 4.49 (dd, *J* = 4.3, 1.7 Hz, 1 H, 3a-H), 4.29 (m, 1 H, 2-H), 3.98 (dd, *J* = 5.8, 1.7 Hz, 1 H, 4-H), 3.76 (ddd, *J* = 6.2, 6.2, 3.4 Hz, 1 H, 5-H), 3.71 (dd, *J* = 12.2, 3.4 Hz, 1 H, 2′a-H), 3.58 (dd, *J* = 12.2, 6.5 Hz, 1 H, 2′b-H), 3.49 (m, 2 H, 1′a-H, 1′b-H), 2.14 (dd, *J* = 14.4, 5.2 Hz, 1 H, 1a-H), 1.72 (ddd, *J* = 14.4, 10.2, 4.9 Hz, 1 H, 1b-H) ppm. ^{13}C NMR (CD_3OD): δ = 169.0, 155.0, 128.2, 125.4, 112.2, 91.0, 86.6, 82.9, 77.9, 77.6, 62.0, 43.1, 36.6 ppm. ***S*-10:** ^{1}HNMR (D_2O), selected signals: δ = 4.49 (dd, *J* = 4.3, 1.7 Hz, 1 H, 3a-H), 4.05 (bd, *J* = 5.2 Hz, 1 H, 4-H), 3.82 (ddd, *J* = 6.5, 5.4, 4.0 Hz, 1 H, 5-H), 3.68 (dd, *J* = 12.1, 3.8 Hz, 1 H, 2′a-H), 3.58 (dd, *J* = 12.1, 6.7 Hz, 1 H, 2′b-H), 3.53 (m, 2 H, 1′a-H, 1′b-H), 2.32 (ddd, *J* = 8.3, 6.2 Hz, 1 H, 1a-H) 1.86 (dd, *J* = 14.4, 5.7 Hz, 1 H, 1b-H) ppm. ^{13}C NMR (CD_3OD): δ = 169.1, 154.9, 128.2, 125.4, 112.2, 92.0, 88.7, 83.5, 79.4, 77.1, 62.3, 44.2, 35.4 ppm.

Compound 24: To a stirred solution of 4-nitro-*N*-[2-(2-naphthyloxy)ethyl]benzensulfonamide[5] (500 mg, 1.344 mmol) and 2,3,4,6-*O*-tetraacetylglucopyranose (608 mg, 1.747 mmol) in dry THF (13.4 mL), PPh_3 (528.8 mg, 2.016 mmol) was added under argon. The reaction mixture was cooled to –80 °C and, after 15 min, DIAD (390.5 μL, 2.016 mmol) was added very slowly. The reaction mixture was stirred at –80 °C for 1 h, and then it was allowed to reach room temperature and stirred overnight. The solvent was evaporated in vacuo, and the product was purified by flash column chromatography (9.5:0.5, toluene/EtOAc) giving **24** (840.5 mg, 84%) as a yellow crystalline solid. ^1H NMR ($CDCl_3$): δ = 8.23, 8.05 (AA′XX′, *J* = 8.6 Hz, 4 H, aromatic protons), 7.73–7.66 (m, 3 H,aromatic protons), 7.42 (bt, *J* = 6.9 Hz, 1 H, aromatic protons), 7.32 (bt, *J* = 7.0 Hz, 1 H, aromatic protons), 7.00 (s, 1 H, aromatic proton), 6.93 (bd, *J* = 8.9 Hz, 1 H, aromatic proton), 5.45 (d, *J* = 8.6 Hz, 1 H), 5.31 (m, 2 H), 5.07 (t, *J* = 9.3 Hz, 1 H), 4.18–4.04 (m, 4 H), 3.82–3.79 (m, 2 H), 3.59–3.54 (m, 1 H), 2.04 (s, 3 H, CH_3), 2.02 (s, 3 H, CH_3), 2.00 (s, 3 H, CH_3), 1.91 (s, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 170.3, 170.0, 169.6, 169.4, 155.8, 150.3, 145.3, 134.5, 129.7, 129.3, 129.0, 127.8, 126.9, 126.8, 124.4, 124.1, 118.5, 107.0, 85.6, 74.5, 73.7, 68.5, 68.0, 66.2, 61.9, 43.8, 21.0 ppm. $[\alpha]_D^{20}$ = –12.8 (*c* = 0.5, DMSO). HRMS (FT-ICR): $C_{32}H_{34}N_2O_{14}SNa$: calcd. 725.1628; found 725.1635 $[M+Na]^+$. $C_{32}H_{34}N_2O_{14}S$ (702.4): calcd. C 54.70, H 4.88, N 3.99; found: C 54.65, H 4.92, N 3.95.

Compound 25: To a stirred solution of **24** (68 mg, 0.096 mmol) in dry CH_3OH (0.5 mL), K_2CO_3 (27 mg, 0.193 mmol) was added under argon at room temperature. After a few minutes, a yellow precipitate appeared and the suspension was stirred at room temperature for 24 h. The reaction mixture was filtered, and the solid residue was dissolved in boiling methanol and then filtered while hot to eliminate salts. The filtrate was concentrated in vacuo and the product was purified by flash column chromatography (8.5:1.5, toluene/CH_3OH) to provide **25**[5] (49 mg, 95%) as a yellow amorphous solid. The resulting product was exclusively the β-anomer.

SCH-54292: The procedure was carried out as described for the preparation of **5**, starting from **25** (40 mg, 0.075 mmol) and, after purification by flash column chromatography (9:1, toluene/CH_3OH), afforded **SCH-54292** (36.5 mg, 93%) as a pale yellow amorphous solid. ^1H NMR (9:1, D_2O/CD_3OD): δ = 7.88 (d, *J* = 7.3 Hz, 1 H, c2-H), 7.86 (d, *J* = 8.8 Hz, 1 H, c3-H), 7.84 (d, *J* = 8.4 Hz, 1 H, c1-H), 7.82, 7.06 (AA′XX′, *J* = 8.8 Hz, 4 H, aromatic protons), 7.53 (t, *J* = 7.6 Hz, 1 H, d-H), 7.43 (t, *J* = 7.5 Hz, 1 H), 7.30 (d, *J* = 2.4 Hz, 1 H, f-H), 7.16 (dd, *J* = 8.9, 2.6 Hz, 1 H, f-H), 5.07 (d, *J* = 9.2 Hz, 1 H, 1-H), 4.33 (m, 2 H, jc-H, jd-H), 3.78 (ddd, *J* = 16.0, 8.0, 5.0 Hz, 1 H, ja-H), 3.67 (t, *J* = 9.0 Hz, 1 H, 2-H), 3.60 (dd, *J* = 12.3, 2.1 Hz, 1 H, 6a-H), 3.59 (ddd, *J* = 16.0, 8.0, 5.0 Hz, 1 H, jb-H), 3.58 (t, *J* = 8.9 Hz, 1 H, 3-H), 3.51 (dd, *J* = 12.3, 5.4 Hz, 1 H, 6b-H), 3.41 (m, 1 H, 5-H), 3.33 (m, 1 H, 4-H) ppm. ^{13}C NMR ([D_6]DMSO): δ = 155.7, 155.4, 134.1, 129.4, 129.2, 128.5, 127.5, 127.4, 126.6, 126.5, 123.8, 118.4, 111.3, 106.6, 86.7, 78.3, 77.2, 70.1, 69.1, 66.2, 60.4, 40.8 ppm. $[\alpha]_D^{20}$ = –42.6 (*c* = 0.5, DMSO). MS: *m*/*z* = 521.2 $[M+H]^+$, 544.3 $[M+H+Na]^+$. HRMS (FT-ICR): $C_{24}H_{28}N_2O_9SNa$: calcd. 543.1413; found 543.1432 $[M+Na]^+$. $C_{24}H_{28}N_2O_9S$ (520.1): calcd. C 55.38, H 5.42, N 5.38; found C 55.58, H 5.89, N 4.76.

NMR Binding Studies

All NMR spectra were recorded at 293 K with a Bruker Avance 500 MHz spectrometer.

Sample Preparation: For the experiments with the free ligand, compounds **9** and **10** were dissolved in a [D_{11}]-Tris buffer at pH = 7.3, containing 100 mM NaCl and 5 mM $MgCl_2$; for compounds **5**–**8**, and **SCH-54292**, 10% CD_3OD was added. COSY, TOCSY, and HSQC experiments were performed by using the standard sequences. A mixing time of 800 ms was employed for NOESY experiments. For the binding experiments, p21 h-Ras, expressed and purified as described previously,[13] was dissolved in 405 μL of the same [D_{11}]-Tris buffer, containing an amount of GDP equimolar to the protein, and transferred into a 5 mm NMR tube; 45 μL of the ligand solution (molecules **5**–**8** and **SCH-54292** dissolved in CD_3OD, molecules **9** and **10** dissolved in D_2O) were added slowly, and the mixture was incubated at 4 °C overnight.

Binding Experiments: trNOESY experiments were carried out without saturation of the residual HDO signal. Optimized mixing times of 200 and 250 ms and molar ratios between 15:1 and 30:1 of compound/protein were employed. In relaxation-edited experiments, T_1 and $T_{1\rho}$ values were calculated for the same ligand protons in the free state and in the presence of p21 h-Ras-GDP complex. Selective ^1H T_1 relaxation times were measured using the standard inversion recovery method with selective pulses at the resonance of interest. $T_{1\rho}$ experiments used a spin-lock period of ca. 4000 Hz. In both cases, 14 different delays were used to monitor the decay of the magnetization. The experiments were repeated for the free molecules and in the presence of Ras-GDP. The relaxation delays for selective T_1 were chosen to cover values between 0.1 and 3 s, while those for $T_{1\rho}$ covered values between 0.05 and 2 s. All the spectra were processed using the program Mestre-C. T_1 and $T_{1\rho}$ values were extracted from fitting the integral of a given proton signal as a function of the relaxation delay, according to a single exponential decay: $y = -a\cdot\exp(-x/b)$, where $b = T_1$ or $T_{1\rho}$. STD experiments were performed without saturation of the residual HDO signal for molar ratios between 15:1 and 50:1 of compound/protein. A train of Gaussian-shaped pulses of 50 ms each was employed, with a total saturation time of the protein envelope of 2 s. MaxB_1 field strength: 50 Hz. An off-resonance frequency of δ = 40 ppm and on-resonance frequencies between δ = 0.8 and –1.5 ppm (protein aliphatic signals region) were applied. In all cases, line broadening

of ligand protons was monitored before and after every binding experiment in order to check our compound's stability.

Biology

Expression and Isolation of Proteins: The C-Cdc25^{Mm} (GEF from mouse; portion of CDC25^{Mm} that contains the catalytic domain of the protein)[13,14] was expressed in *Escherichia coli* using the pGEX-2T expression vector, and was affinity-purified by using Glutathione Sepharose 4B resin. p21 h-Ras was expressed in *E. coli* using the pQE-30 expression vector, and was affinity-purified as a 6×His-tagged protein by using Ni-NTA resin.[13]

Measurement of C-Cdc25^{Mm}-Stimulated Guanine Nucleotide Exchange on p21 h-Ras: To investigate the ability of putative Ras inhibitors to inhibit or to reduce the C-Cdc25^{Mm}-stimulated nucleotide exchange on purified human Ras proteins, we used a technique described by Lenzen et al.[10] with some modifications. This approach utilizes guanine nucleotides carrying an *N*-methylanthraniloyl fluorophore (MANT-GDP or MANT-GTP). p21 h-Ras (100 nM) and MANT-GTP (0.5 μM) were incubated in buffer B (40 mM Hepes, pH = 7.5, 2 mM DTT, 100 μM $MgCl_2$) in the absence and in the presence of the inhibitors. The exchange reaction was started by the addition of C-Cdc25^{Mm} (25 nM), and then monitored at an excitation wavelength of 370 nm and an emission wavelength of 450 nm with a Perkin–Elmer Luminescence Spectrometer. Measurements were taken every second. The slope of each curve was calculated.

Measurement of Dissociation Rate: We used the method described by Lenzen et al.[10] with some modifications to investigate the ability of Ras inhibitors to influence the C-Cdc25^{Mm}-stimulated dissociation rate of p21 h-Ras·MANT-GDP complexes. The complex p21 h-Ras·MANT-GDP (200 nM), obtained as described by Lenzen et al.[10], and an excess of GDP (500 μM) were incubated in buffer B (40 mM Hepes, pH = 7.5, 2 mM DTT, 100 μM $MgCl_2$) in the absence and in the presence of the inhibitors. The dissociation reaction was started by addition of C-Cdc25^{Mm} (100 nM), and then monitored at an excitation wavelength of 370 nm and an emission wavelength of 450 nm with a Perkin–Elmer Luminescence Spectrometer. Measurements were taken every second. The slope of each curve was calculated assuming a single exponential decay.

Cell Cultures and Growth Conditions: Normal NIH3T3 and k-Ras (Arg12) transformed[11] mouse fibroblasts (from Dr. P. Bossu, Dompé, L'Aquila, Italy) were grown on 60 mm plastic dishes in Dulbecco's modified Eagle's medium supplemented with 10% newborn calf serum (Gibco), penicillin (100 units/mL), and streptomycin (100 μg/mL). Cells were detached by treating them with 0.05% trypsin and EDTA (0.15 mM), and growth was monitored by counting the cell number/mL with a Coulter Counter ZM.

Assay of MAPK Activation: Cells were scraped, and ice-cold Lysis buffer (25 mM HEPES, pH = 7.5, 150 mM NaCl, 1% NP-40, 0.25% Na deoxycholate, 10% glycerol, 25 mM NaF, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM Na vanadate, one tablet of Protease Inhibitor Mixture from Roche Applied Science in 50 mL of extraction medium) was added. The lysates were transferred to a microcentrifuge tube on ice and centrifuged. Proteins were separated by SDS-PAGE, transferred to nitrocellulose, and immunodecorated with anti-p42/44 MAPK antibody and anti-phospho-p42/44 MAPK antibody (Cell Signalling Technology, Beverly, MA, USA). Bound antibodies were revealed with the ECL Western blotting analysis system (Amersham Pharmacia Biotech).

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