Nucleosides XI.[1] Synthesis and Antiviral Evaluation of 5′-Alkylthio-5′-deoxy Quinazolinone Nucleoside Derivatives as *S*-Adenosyl-L-homocysteine Analogs

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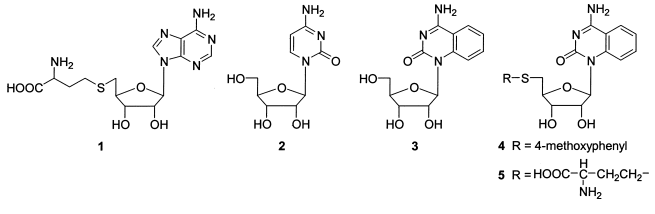
4-Amino-1-(β-D-ribofuranosyl)quinazolin-2-one (3) was prepared by a direct glycosylation of 4-aminoquinazolin-2-one (7) using the Vorbruggen's silylation method and provided exclusively the β-anomer. This quinazoline nucleoside and its 2′,3′-*O*-isopropylidene derivative (9) did not undergo the coupling reaction with dialkyl disulfides in the presence of tri-*n*-butylphosphine unless their 4-amino groups were protected by *N*,*N*-dimethylaminomethylidene. This approach provides a viable alternative synthetic route to 5′-alkylthio-5′-deoxy nucleosides.

Key words quinazoline; tri-*n*-butylphosphine; AdoHcy; *S*-adenosyl-L-homocysteine

Several nucleosides containing 5′-sulfur substituents have been discovered in nature and are involved in many important biological processes. One of the most significant processes is the *S*-adenosyl-L-methionine (AdoMet)-dependent transmethylations. Inhibition of these methyl transfer processes has been correlated with antiviral activity and has attracted considerable attention as a target for the discovery of new antiviral agents.[3,4] *S*-Adenosyl-L-homocysteine (**1**, AdoHcy), the byproduct of these AdoMet-dependent transmethylations, and some of its structural analogs have been shown to be potent competitive product inhibitors of the AdoMet-dependent methyltransferases.[5—15] Our group has also been interested in the synthesis and biological evaluation of several 5′-sulfur containing nucleosides.[16—18] In continuation of our studies and our longstanding interest in the biological activities of quinazoline derivatives,[19] we initiated an investigation on the synthesis and biological evaluation of 5′-sulfur containing quinazoline nucleoside derivatives.

Quinazoline nucleosides were first synthesized by Stout and Robins in 1968 as pyrimidine nucleoside analogs[20] and consequent synthetic studies were contributed by Dunkel and Pfleiderer in the 1990s.[21—23] Quinazoline nucleosides were once used as a conformationally restricted model to study the *syn–anti* conformational preference of pyrimidine nucleosides in solution.[24,25] More recently, quinazoline nucleosides have been incorporated into oligonucleotides as pyrimidine nucleoside substitutes to study the binding affinity and base-pairing selectivity.[26] However, their biological activities are still relatively less known in literature. 4-Amino-1-(β-D-ribofuranosyl)quinazolin-2-one (**3**) was chosen for our study. This quinazoline nucleoside was previously synthesized from quinazoline-2,4-dione by Stout and Robins as a cytidine (**2**) analog,[20] and subsequently shown to display the antiviral activity against herpes simplex virus.[27] Other derivatives from this nucleoside have rarely been studied.[20,21] In an effort to explore the antivirial profiles and the chemical properties of the 4-aminoquinazolin-2-one nucleoside (**3**), two target structures containing 5′-sulfur substituents (**4**, **5**) were designed as *S*-adenosyl-L-homocysteine (**1**, AdoHcy) analogs. Herein, we report an improved synthesis of **3** and the synthesis of 5′-alkylthio-5′-deoxy quinazolinone nucleosides **4** and **5**.



Results and Discussion

A perusal of the literature revealed that most of 4-aminoquinazolin-2-one nucleosides were synthesized by ribosylation of quinazoline-2,4-diones followed by functional group interconversions.[20,21] Our first effort was to develop a direct synthetic route from 4-aminoquinazolin-2-one[28,29] (**7**). The heterocycle **7** was prepared *via* a 1,3-dicyclohexylcarbodiimide (DCC)—mediated cyclodesulfurative annulation reaction developed in our laboratory.[30,31] Anthranilamide (**6**) was condensed with benzoyl isothiocyanate to form the thiourea intermediate which was immediately treated with DCC followed by ammonia to afford 4-aminoquinazolin-2-one (**7**) in 88% yield. Compound **7** was coupled with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose under modified Vorbruggen's condition.[32,33] This reaction gave exclusively one anomer of the sugar-protected ribonucleoside **8** which was subsequently deprotected with ammonia to afford 4-amino-1-(β-D-ribofuranosyl)quinazolin-2-one (**3**) in 74% yield (Chart 1).

The 2′,3′-diol of **3** was protected with acetone in the presence of acid to form the acetonide **9** in 74% yield. The structural elucidation was carried out by intensive NMR studies. The chemical shift difference of the two isopropylidene methyl groups ($\Delta\delta$) in **9** was 0.22 which indicates a β-anomeric configuration based on Imbach's empirical rule.[34] The 1-D NOE irradiation of **3** and **9** at 1′-H (δ 6.12, 6.23) showed NOE enhancements at one of the aromatic protons (δ 7.64, 7.57, assigned as 8-H) and *vice versa*. These results suggested that the ribosylation of **7** was carried out at the N^1-position. The NOE study also revealed that **3** and **9** exist in the *syn* conformation. This observation is consistent with M. P. Schweizer's studies[24,25] (Table 1).

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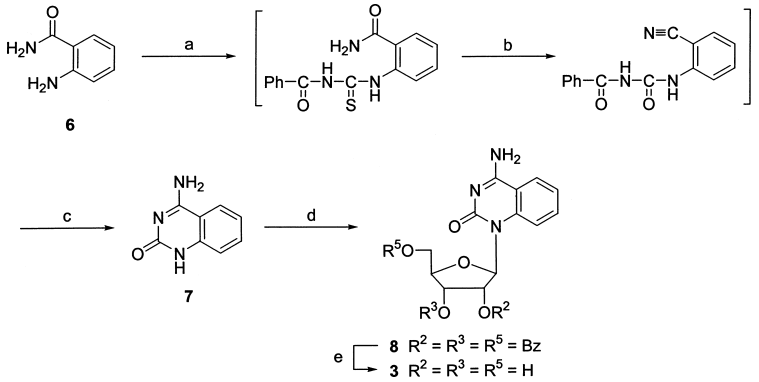
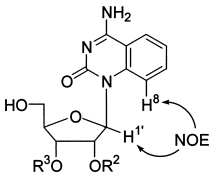


Reagents and conditions: (a) benzoyl isothiocyanate, DMF, r.t.; (b) DCC, r.t.; (c) NH_4OH/MeOH/DMF, r.t., 88% from **6**; (d) (i) BSTFA, CH_3CN, 75 °C, 20 min; (ii) 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose, TMSOTf, CH_3CN, 75 °C, 1 h; (e) aq. NH_4OH/MeOH/acetone, r.t., 26 h, 74% from **7**.

Chart 1

Table 1. NOE Effects of Compounds **3** and **9**



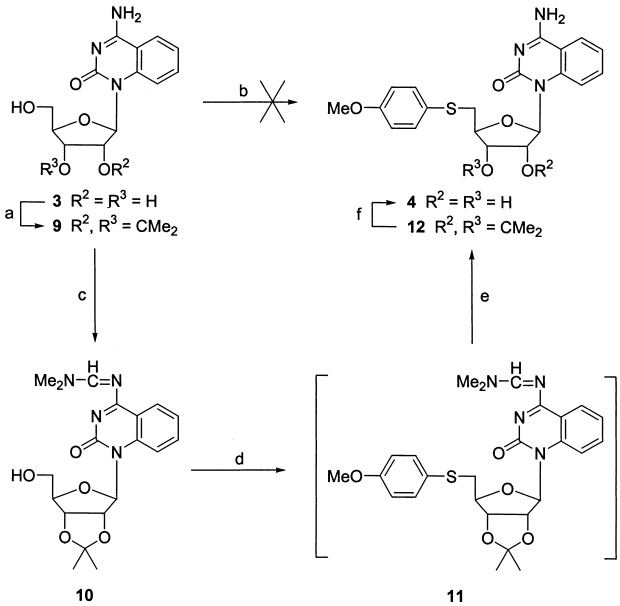
Compound	Proton	Chemical shift	NOE effect	
			Irradiated at 1′-H	Irradiated at 8-H
3 $R^2=R^3=H$	8-H	7.64 (m)	2.70%	
	1′-H	6.12 (d)		4.35%
9 R^2, $R^3=CMe_2$	8-H	7.57 (m)	1.27%	
	1′-H	6.23 (s)		1.20%

A convenient preparation of 5′-arylthio-5′-deoxynucleosides was reported by T. Hata *et al.* involving the reaction of nucleosides with dialkyl disulfides in the presence of tri-*n*-butylphosphine in pyridine.[5,9] In our attempt to synthesize the 5′-arylthio-5′-deoxy 4-aminoquinazolin-2-one nucleoside (**4**), **3** or **9** was treated with bis(4-methoxyphenyl) disulfide under Hata's condition, in the presence of tri-*n*-butylphosphine in pyridine, but no desired product (**4** or **12**) was observed in the reaction.

Nevertheless, **9** was first treated with *N*,*N*-dimethylformamide dimethyl acetal to protect the amino group at the 4-position, then reacted with bis(4-methoxyphenyl) disulfide under Hata's condition to form the desired product **11**. Compound **11**, without purification, was immediately treated with ammonium hydroxide to remove the protecting group to give 4-amino-1-[5-deoxy-5-((4-methoxyphenyl)thio)-2,3-*O*-isopropylidene-*β*-D-ribofuranosyl]quinazolin-2-one (**12**) in 62% yield after 3 steps. The isopropylidene was removed by treatment of **12** with 90% aqueous trifluoroacetic acid to afford the desired product **4** in 71% yield (Chart 2).

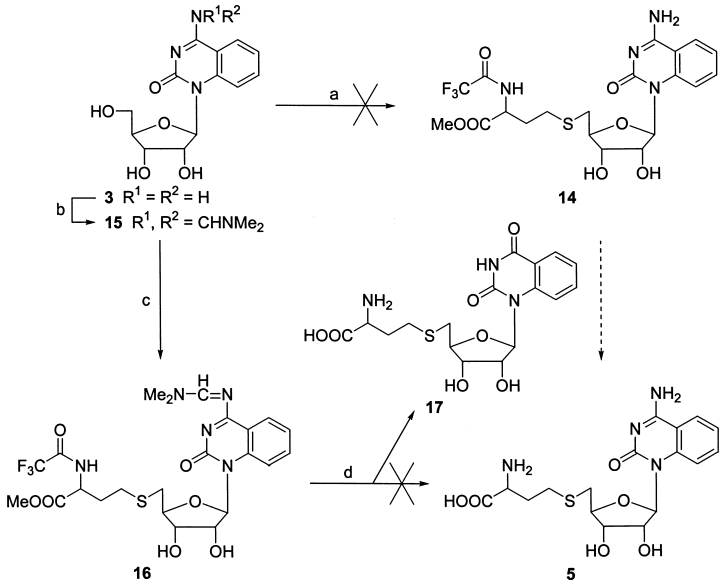
This result indicated that the amino group at the 4-position of quinazoline played an important role in the reaction. It is noticeable that the conversion of cytidine (**2**) to 5′-arylthio-5′-deoxycytidines under a similar condition has previously been reported by T. Hata *et al.*[5,9] However, the quinazoline nucleosides **3** and **9** did not undergo the similar reaction as cytidine under the same condition unless the amino group at the 4-position of quinazoline was protected with dimethylaminomethylidene. Furthermore, the formation of O^2,5′-cyclic nucleosides by a Mitsunobu-type dehydration reaction was not observed in the reaction.[35]

The success in the synthesis of **4** prompted us to broaden the scope of this strategy. P. Serafinowski *et al.* have demonstrated that a suitably protected L-homocystine can be condensed with unprotected nucleosides under Hata's condition to give the protected AdoHcy analogs.[11,12] The direct condensation of **3** with *N*,*N*-bis(trifluoroacetyl)-L-homocystine dimethyl ester[11,13] (**13**) in the presence of tri-*n*-butylphosphine proved unsuccessful as expected. However, with the amino-protected quinazoline nucleoside (**15**) prepared from



Reagents and conditions: (a) $HC(OEt)_3$, *p*-TsOH·H_2O, acetone, r.t., 6 h, 74%; (b) bis(4-methoxyphenyl) disulfide, 10 eq *n*-Bu_3P, pyridine; (c) *N*,*N*-dimethylformamide dimethyl acetal, DMF, r.t., 24 h; (d) bis(4-methoxyphenyl) disulfide, *n*-Bu_3P, pyridine, r.t., 24 h; (e) NH_4OH/MeOH, 62% from **9**; (f) TFA/H_2O (9 : 1, v/v), r.t., 90 min, 71%.

Chart 2



Reagents and conditions: (a) *N*,*N*-bis(trifluoroacetyl)-L-homocystine dimethyl ester (**13**), *n*-Bu_3P, pyridine/DMF, r.t.; (b) *N*,*N*-dimethylformamide dimethyl acetal, DMF, r.t., 24 h; (c) *N*,*N*-bis(trifluoroacetyl)-L-homocystine dimethyl ester (**13**), *n*-Bu_3P, pyridine, r.t., 7 d, 5% from **3**; (d) (i) $Ba(OH)_2$, H_2O/MeOH; (ii) H^+, 50% from **16**.

Chart 3

3, the coupling reaction proceeded but only gave 5% of the desired product **16** accompanied with unreacted starting materials. The dialkyl disulfides are generally much less reactive than diaryl disulfides in the phosphine-mediated coupling reaction[9,11,12,36] which resulted in the low reaction yield.

Treatment of **16** with aqueous barium hydroxide followed by neutralization with acid gave the fully deprotected product **17** which was surprisingly not the desired product **5**. EI-MS (electron impact mass spectrometry) analysis (70 eV) of **17** showed strong fragment peaks at *m*/*z* 64 (18%), 92 (49%), 119 (85%), and 162 (100%). This result is consistent with the fragment peaks of quinazoline-2,4-dione, which suggested a quinazoline-2,4-dione nucleoside derivative was obtained after the hydrolysis. ESI-HR-MS (electrospray ionization) (formic acid) confirmed the proposed structure of **17**. UV–Vis absorption spectroscopy also supported the assignment. The literature revealed that the λ_{max} of **3** displays significant difference at pH 1 [326 nm (ε 4700)] and pH 11 [317 nm (ε 4500)] while the λ_{max} of the quinazoline-2,4-dione analog, 1-(β-D-ribofuranosyl)quinazoline-2,4-dione, was not affected by pH [pH 1: 306 nm (ε 3900); pH 7: 304 nm (ε 3715); pH 11: 306 nm (ε 4200)].[20,21] Compound **17** shows λ_{max} at 306 nm (ε 3433, pH=1), 305 nm (ε 3830, MeOH/H_2O), and 307 nm (ε 2542, pH=11). Therefore, the structure of **17** was identified unambiguously as *S*-[1-(quinazoline-2,4-dion-1-yl)-β-D-ribofuranosyl]-L-homocysteine (Chart 3). This unexpected displacement of the *N*,*N*-dimethylformamidinyl group has not been reported in the literature.

Conclusion

In summary, our investigation has provided an alternative route for the synthesis of 5′-alkylthio-5′-deoxy nucleosides. The use of *N*,*N*-dimethylaminomethylidene to protect the amino group on the nucleoside bases can reduce the participation of base-functionalities in the reaction which was carried out at the 5′-position. The nucleoside derivatives **4** and **17** were screened for antiviral activities against herpes simplex virus type-1 (HSV-1) and Epstein-Barr virus (EBV) and showed no activity at non-cytotoxic concentrations. The unexpected hydrolysis of the *N*,*N*-dimethylformamidinyl group provides an alternative interconversion of functionalities. Its synthetic application for other quinazoline derivatives is in progress.

Experimental

General Chemical Procedures Melting points were obtained on an Electrothermal apparatus and are uncorrected. NMR spectra were obtained on Varian Gemini-300, JEOL JNM-EX400 or Bruker DRX500 spectrometer. IR spectra were recorded on a Jasco A-100 infrared spectrophotometer. Mass spectra were recorded on Finnigan TSQ-46C (EI), JEOL JMS-D300 (EI), VG 70-250S (FAB) or Micromass LCT (ESI) mass spectrometer. Elemental analyses for C, H, and N were carried out either on a Heraeus elemental analyzer or Perkin-Elmer 240 elemental analyzer and were within $\pm$0.4% of the theoretical values. Thin-layer chromatography (TLC) was performed on Merck plates precoated with silica gel 60 containing fluorescent indicator. Compounds on thin-layer chromatography were visualized by illumination under UV light (254 nm), or dipped into 10% methanolic sulfuric acid followed by charring on a hot plate. Solvent systems are expressed as a percentage of the more polar component with respect to total volume (v/v%). Merck silica gel (230—400 mesh) was used for flash column chromatography as described by Still, W. C. *et al.*[37] Evaporation was carried out with a rotary evaporator under reduced pressure with the bath temperature below 50 °C unless specified otherwise. Materials obtained from commercial suppliers were used without further purification. The reported yields have not been optimized.

4-Aminoquinazolin-2-one (7) To a solution of anthranilamide (**6**, 5.00 g, 36.72 mmol) in anhydrous *N*,*N*-dimethylformamide (DMF) (50 ml) was added benzoyl isothiocyanate (5.9 ml, 7.16 g, 43.89 mmol, 1.2 eq). After the reaction mixture was stirred at room temperature under nitrogen for 2 h, dicyclohexylcarbodiimide (DCC) (16.69 g, 80.89 mmol, 2.2 eq) was added and the mixture was stirred at room temperature for an additional 24 h. Acetone (40 ml) was added to the mixture and the solution was vigorously stirred for 20 min. The precipitated solid was collected by filtration and washed with chloroform. The solid was then dissolved in a mixture of methanol/28% concentrated ammonium hydroxide solution/DMF (50 ml : 50 ml : 100 ml) and the reaction mixture was stirred at room temperature for 24 h. The solvents were removed under reduced pressure to dryness. Methanol (30 ml) was added to the resulting oil and the mixture was

vigorously stirred for 1 h. The precipitated solid was collected by filtration and washed with chloroform to give **7** (8.59 g, 32.3 mmol, 88%). An analytical sample of **7** was recrystallized from EtOH/H_2O. mp >360 °C (dec.) (EtOH/H_2O) [lit.[29] >360 °C (DMF–EtOH)]; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 7.05—7.13 (m, 2H, Ph), 7.52—7.57 (m, 1H, Ph), 7.78 (br s, 1H, NH), 7.85 (br s, 1H, NH), 7.96—7.99 (d, 1H, Ph), 10.67 (br s, 1H, NH); MS (EI, 70 eV) *m/z* 118 (76), 161 (100) (M^+); MS (ESI, formic acid) *m/z* 162 (100) (M^++1); HR-MS (EI) Calcd for $C_8H_7N_3O$: 161.0589, Found: 161.0590; *Anal.* Calcd for $C_8H_7N_3O \cdot 0.5H_2O$: C, 56.46; H, 4.74; N, 24.69. Found: C, 56.59; H, 4.83; N, 24.92.

4-Amino-1-(β-D-ribofuranosyl)quinazolin-2-one (3) To a solution of **7** (5.00 g, 31.06 mmol) in acetonitrile (100 ml) was added bis(trimethylsilyl)trifluoroacetamide (BSTFA) (33.0 ml, 31.98 g, 124.23 mmol, 4 eq) and the reaction mixture was stirred at 75 °C for 25 min. This solution was treated with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (19.00 g, 37.70 mmol, 1.21 eq) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) (9.0 ml, 10.35 g, 46.57 mmol, 1.5 eq) and then stirred at 75 °C for an additional 1 h. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure to dryness. The residue was dissolved in a mixture of methanol/acetone/28% concentrated ammonium hydroxide solution (200 ml : 100 ml : 200 ml) and the reaction mixture was stirred at room temperature for 30 h. The solvents were removed under reduced pressure. The resulting oil-like residue was filtered and the collected solid was washed with cold ethanol to give the first portion of the product. The filtrate was concentrated to dryness. Acetone (40 ml) was added to the resulting oil and the mixture was vigorously stirred for 30 min. The precipitated solid was collected by filtration and washed with cold ethanol to give the second portion of the product (**3**, 6.78 g overall, 23.11 mmol, 74%). An analytical sample of **3** was obtained by recrystallization from EtOH/H_2O. mp 246—247 °C (dec.) (EtOH/H_2O) [lit.[20] 259—260 °C (dec.)]; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 3.52—3.78 (m, 3H, 4′ and 5′-H), 4.13 (dd, 1H, *J*=12.4, 6.1 Hz), 4.53 (dd, 1H, *J*=12.2, 6.0 Hz), 4.93—4.96 (m, 2H, 2×OH), 5.11 (d, 1H, *J*=5.9 Hz, OH), 6.12 (d, 1H, *J*=5.7 Hz, 1′-H), 7.18—7.23 (m, 1H, Ph), 7.61—7.66 (m, 2H, Ph), 7.96—8.06 (m, 3H, Ph and NH_2); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 61.9 (5′-CH_2), 69.6 (2×CH), 84.7 (CH), 90.8 (1′-CH), 110.8, 116.1 (CH), 122.0 (CH), 125.2 (CH), 134.0 (CH), 142.3, 155.7, 163.4; MS (FAB) *m/z* 137, 154, 162 (100), 185, 294 (42) (M^++1); *Anal.* Calcd for $C_{13}H_{15}N_3O_5 \cdot 0.5H_2O$: C, 51.65; H, 5.34; N, 13.90. Found: C, 51.95; H, 5.15; N, 13.72.

4-Amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)quinazolin-2-one (9) Compound **3** (1.98 g, 6.76 mmol) was suspended in acetone (40 ml) containing *p*-toluenesulfonic acid monohydrate (1.50 g, 7.89 mmol, 1.17 eq). Triethyl orthoformate (4.0 ml, 3.56 g, 24.04 mol, 3.6 eq) was added at room temperature with vigorous stirring. After all the solid dissolved, the reaction mixture was stirred for an additional 4 h, and then the solution was adjusted to pH 8 with diluted ammonia water. The solution was concentrated to 20 ml under reduced pressure. The aqueous solution was extracted with ethyl acetate (3×50 ml). The organic layer was combined and the solvent was evaporated to give the crude product of **9** as a white solid (1.67 g, 5.02 mmol, 74%). The solid was recrystallized from ethanol to give **9** (0.6750 g, 2.03 mmol, 30%). mp 271—272 °C (EtOH); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 1.29 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 3.53—3.58 (m, 1H, 5′-H), 3.63—3.67 (m, 1H, 5′-H), 4.02 (dd, 1H, *J*=9.7, 5.2 Hz), 4.91—4.94 (m, 2H, including one D_2O exchangeable proton, 5′-OH), 5.26 (dd, 1H, *J*=6.6, 2.1 Hz), 6.23 (s, 1H, 1′-H), 7.23—7.26 (m, 1H, Ph), 7.56—7.57 (m, 1H, Ph), 7.64—7.67 (m, 1H, Ph), 8.05—8.21 (m, 3H, 1×Ph and NH_2); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 26.0 (CH_3), 28.0 (CH_3), 62.5 (5′-CH_2), 82.2 (CH), 83.5 (CH), 88.2 (CH), 91.1 (1′-CH), 111.0, 113.8, 115.7 (CH), 122.8 (CH), 126.0 (CH), 134.9 (CH), 142.7, 155.6, 164.2; MS (FAB) *m/z* 162 (100), 334 (20) (M^++1); HR-MS (FAB) Calcd for $C_{16}H_{20}N_3O_5$: 334.1403, found: 334.1402; *Anal.* Calcd for $C_{16}H_{19}N_3O_5$: C, 57.65; H, 5.75; N, 12.61. Found: C, 57.61; H, 5.70; N, 12.67.

4-(*N*,*N*-Dimethylformamidinyl)-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)quinazolin-2-one (10) To a suspension of **9** (2.36 g, 7.08 mmol) in DMF (47 ml) was added *N*,*N*-dimethylformamide dimethyl acetal (3.8 ml, 3.40 g, 28.51 mmol, 4 eq). The reaction mixture was stirred at room temperature under nitrogen for 24 h. The solvent was evaporated *in vacuo* to give the crude product of **10**. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.35 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 3.23 (s, 3H, CH_3), 3.27 (s, 3H, CH_3), 3.83—4.03 (m, 3H, 5′-H and OH), 4.35 (dd, 1H, *J*=6.3, 3.0 Hz), 5.29 (dd, 1H, *J*=6.0, 4.4 Hz), 5.48 (dd, 1H, *J*=6.5, 2.3 Hz), 6.21 (d, 1H, *J*=2.3 Hz, 1′-H), 7.19—7.24 (m, 1H, Ph), 7.46—7.49 (m, 1H, Ph), 7.60—7.66 (m, 1H, Ph), 8.38—8.41 (m, 1H, Ph), 8.93 (s, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 25.8 (CH_3), 27.9 (CH_3), 36.1 (CH_3), 42.2 (CH_3), 63.7 (5′-CH_2), 81.2, 84.5, 88.8, 92.4 (1′-CH), 113.5, 114.2, 116.9, 122.9, 128.8, 135.0, 143.6, 157.2, 159.3, 170.1 (–CH=N).

4-Amino-1-[5-deoxy-5-((4-methoxyphenyl)thio)-2,3-*O*-isopropylidene-β-D-ribofuranosyl]quinazolin-2-one (12) To the crude product **10** and bis(4-methoxyphenyl)disulfide (4.93 g, 17.73 mmol, 2.5 eq) in dry pyridine (35 ml) was added tri-*n*-butylphosphine (24.4 ml, 3.57 g, 17.66 mmol, 2.5 eq) *via* a syringe. The reaction was stirred for 24 h at room temperature under nitrogen, then the solvent was evaporated under reduced pressure. Hexane (50 ml) was added to the resulting oil and the mixture was stirred vigorously at room temperature for 15 min. The hexane layer was decanted and the resulting oil was concentrated to dryness. The resulting oil was treated with 28% concentrated ammonium hydroxide/methanol (140 ml, 1 : 1, (v/v)) and the mixture was stirred at room temperature for 24 h. The solvents were evaporated under reduced pressure and the resulting oil was purified by flash column chromatography ($CHCl_3$/MeOH=97.5 : 2.5) to give **12** (2.00 g, 4.40 mmol, 62% from **9**, *Rf*=0.23 ($CHCl_3$/MeOH=95 : 5)). An analytical sample of **12** was obtained by recrystallization from MeOH. mp 123 °C (MeOH); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 1.28 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 3.15 (dd, 1H, *J*=13.7, 6.6 Hz, 5′-H), 3.24 (dd, 1H, *J*=13.7, 7.4 Hz, 5′-H), 3.70 (s, 3 H, CH_3), 4.04—4.09 (m, 1H), 4.94 (dd, 1H, *J*=6.2, 3.1 Hz), 5.34 (d, 1H, *J*=6.5 Hz), 6.07 (s, 1 H, 1′-H), 6.81 (d, 2H, *J*=8.6 Hz, Ph), 7.22—7.27 (m, 1H, Ph), 7.31 (d, 2H, *J*=8.6 Hz, Ph), 7.49—7.52 (m, 1H, Ph), 7.64—7.69 (m, 1H, Ph), 8.06—8.14 (m, 3H, Ph and NH_2); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 25.3 (CH_3), 27.2 (CH_3), 37.6 (5′-CH_2), 55.5 (CH_3), 84.1, 84.7, 87.4, 91.4 (1′-CH), 110.5, 112.7, 114.7, 115.0, 122.3, 125.4, 125.6, 132.6, 134.6, 142.5, 154.9, 158.7, 163.7; MS (EI/20 eV) *m/z* 100, 194, 294 (100), 455 (28) (M^+); HR-MS (FAB) Calcd for $C_{23}H_{26}N_3O_5S$: 456.1593, Found: 456.1592; *Anal.* Calcd for $C_{23}H_{25}N_3O_5S \cdot 0.5CH_3OH$: C, 59.86; H, 5.77; N, 8.91. Found: C, 60.09; H, 5.67; N, 8.91.

4-Amino-1-[5-deoxy-5-((4-methoxyphenyl)thio)-β-D-ribofuranosyl] quinazolin-2-one (Trifluoroacetic Acid Salt) (4) Compound **12** (0.59 g, 1.30 mmol) was dissolved in a 90% aqueous trifluoroacetic acid solution (13 ml) and the mixture was stirred at room temperature for 90 min. The solvents were removed under reduced pressure and the resulting solid was recrysallized from ethanol to give **4** (0.49 g, 0.92 mmol, 71%). mp 175—177 °C (dec.) (EtOH); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 3.14 (dd, 1H, *J*=13.8, 7.4 Hz, 5′-H), 3.32 (dd, 1H, *J*=13.8, 4.3 Hz, 5′-H), 3.73 (s, 3H, CH_3), 3.87 (dd, 1H, *J*=11.0, 6.8 Hz), 4.22 (t, 1H, *J*=6.3 Hz), 4.63 (t, 1H, *J*=5.5 Hz), 6.03 (d, 1H, *J*=4.6 Hz, 1′-H), 6.88 (d, 2H, *J*=8.7 Hz, 4-MeOPh), 7.36 (d, 2H, *J*=8.7 Hz, 4-MeOPh), 7.41—7.44 (m, 1H, Ph), 7.64—7.66 (m, 1H, Ph), 7.84—7.87 (m, 1H, Ph), 8.30—8.32 (m, 1H, Ph), 9.40—9.85 (br s, 2H, NH_2); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 38.2 (5′-CH_2), 56.0 (CH_3), 70.8 (CH), 72.8 (CH), 82.8 (CH), 92.0 (1′-CH), 110.2, 115.6 (CH), 117.1 (CH), 117.3 (q, *J*=294 Hz, CF_3 of TFA), 124.6 (CH), 126.8, 127.2 (CH), 133.0 (CH), 137.8 (CH), 141.8, 149.3, 159.2, 159.8 (q, *J*=34 Hz, C=O of TFA), 160.3; MS (ESI, formic acid) *m/z* 162 (100), 255 (58), 416 (53) (M^++1); HR-MS (ESI) Calcd for $C_{20}H_{22}N_3O_5S$: 416.1280, Found: 416.1284; *Anal.* Calcd for $C_{20}H_{21}N_3O_5S \cdot CF_3COOH$: C, 49.90; H, 4.19; N, 7.94. Found: C, 49.96; H, 4.23; N, 7.86.

***N*,*N*-Bis(trifluoroacetyl)-L-homocysteine Dimethyl Ester (13). [Method A][11]** L-Homocystine (4.74 g, 17.67 mmol) was suspended in anhydrous methanol (30 ml) and cooled to 0 °C. Dry hydrogen chloride gas was bubbled through the suspension till the solution became clear (approximately 30 min). Dimethyl sulfite (11.55 g, 104.9 mmol, 5.9 eq) was added to the solution and the mixture was heated at 60 °C for 1 h. The solvent was removed under reduced pressure. The resulting residue was dissolved in trifluoroacetic anhydride (17.17 g, 81.75 mmol, 4.6 eq) and the reaction mixture was stirred at 60 °C for 1 h. The solvent was removed under reduced pressure, and the residue was dissolved in EtOAc (100 ml), washed with saturated $NaHCO_3$ solution (50 ml), and saturated NaCl solution (50 ml), then dried over anhydrous Na_2SO_4 and evaporated to dryness. The resulting residue was purified by column chromatography (Hex/EtOAc=75 : 25, *Rf*=0.11) to give **13** (4.11 g, 8.41 mmol, 48%).

[Method B] L-Homocystine (5.00 g, 18.63 mmol) was suspended in anhydrous methanol (100 ml) and cooled to −78 °C. To the suspension was added thionyl chloride (10 ml, 16.38 g, 137.67 mmol, 7.39 eq) dropwise. The reaction temperature was allowed to warm to room temperature and stirred for an additional 16 h. The solvent was removed under reduced pressure, and the residue was dried under reduced pressure. The residue was dissolved in trifluoroacetic anhydride (25 ml, 37.18 g, 0.177 mol, 9.5 eq) and the reaction mixture was stirred at 60 °C for 1.5 h. The solvent was removed under reduced pressure, and the residue was dissolved in ethyl actate (100 ml), washed with saturated $NaHCO_3$ solution (2×60 ml), and saturated NaCl solution (60 ml), dried over anhydrous Na_2SO_4 and then evaporated to dryness.

The resulting residue was purified by flash column chromatography (Hex/EtOAc=7 : 3, *Rf*=0.24) to give **13** (8.59 g, 17.60 mmol, 95%). An analytical sample of **13** was obtained by recrystallization from methanol. mp 87—89 °C (MeOH) [lit.[11] 89—90 °C (MeOH/H_2O)]; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.12 (m, 2H, β-H), 2.29 (m, 2H, β-H), 2.65 (dt, 4H, J=7.0, 4.3 Hz, γ-H), 3.73 (s, 6H, OCH_3), 4.68 (dt, 2H, J=8.0, 5.1 Hz, α-H), 7.70 (d, 2H, J=8.0 Hz, NH); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 31.3, 33.8, 51.4, 52.8 (CH_3), 115.2 (q, J=287 Hz, CF_3), 156.7 (q, J=37 Hz, CF_3–C=O), 170.4 (C=O); MS (EI/11 eV) *m/z* 212, 488 (100) (M^+).

Methyl *N*-Trifluoroacetyl-*S*-[1-(4-(*N*,*N*-dimethylformamidinyl)quinazolin-2-on-1-yl)-β-D-ribofuranosyl]-L-homocysteine (16) To a suspension of **3** (0.59 g, 2.00 mmol) in DMF (12 ml) was added *N*,*N*-dimethylformamide dimethyl acetal (1.1 ml, 0.95 g, 8.00 mmol, 4 eq). The reaction mixture was stirred at room temperature under nitrogen for 24 h. The solvent was evaporated *in vacuo* to give the *N*-protected product **15**. To **15** and the protected amino acid **13** (2.75 g, 5.60 mmol, 2.8 eq) in dry pyridine (10 ml) was added tri-*n*-butylphosphine (3.0 ml, 2.43 g, 12.00 mmol, 6 eq) *via* a syringe. The reaction was stirred for 7 d at room temperature under nitrogen. Methanol (10 ml) was added and the solvents were evaporated under reduced pressure. Hexane (30 ml) was added to the residue and the mixture was stirred vigorously at room temperature for 10 min. The hexane layer was decanted and the resulting oil was purified by flash column chromatography ($CHCl_3$/MeOH=95 : 5) to give **16** (oil, 56 mg, 0.097 mmol, 5%, *Rf*=0.24 ($CHCl_3$/MeOH=9 : 1)). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.10—2.19 (m, 2H), 2.59—2.67 (m, 2H), 2.92 (dd, 1H, J=14.0, 6.5 Hz, 5′-H), 3.01 (dd, 1H, J=14.0, 4.7 Hz, 5′-H), 3.23 (s, 3H, CH_3), 3.26 (s, 3H, CH_3), 3.68 (s, 3H, CH_3), 4.03—4.11 (m, 1H, α-H), 4.61 (dd, 1H, J=6.5, 2.8 Hz), 4.70 (t, 1H, J=7.0 Hz), 4.89 (dd, 1H, J=6.5, 2.7 Hz), 6.14 (d, 1H, J=2.8 Hz, 1′-H), 7.18—7.23 (m, 1H, Ph), 7.48—7.51 (m, 1H, Ph), 7.58—7.64 (m, 1H, Ph), 7.72 (d, 1H, J=7.1 Hz, α-NH), 8.35—8.38 (m, 1H, Ph), 8.78 (s, 1H, –CH=N); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 29.4, 31.5, 34.9, 36.0, 42.2, 52.8, 53.4, 72.7, 73.4, 83.8, 92.9, 114.5, 117.0, 122.9, 128.7, 134.8, 143.4, 157.1, 159.1, 169.9, 171.5 (without CF_3 and CF_3–C=O); MS (FAB) *m/z* 136, 154 (100), 219, 307, 418, 576 (10) (M^++1); HR-MS (FAB) Calcd for $C_{23}H_{29}N_5O_7F_3S$: 576.1740, Found: 576.1740.

***S*-[1-(Quinazoline-2,4-dion-1-yl)-β-D-ribofuranosyl]-L-homocysteine (17)** A solution of **16** (56 mg, 0.097 mmol) in methanol (10 ml) was diluted with 0.5 M barium hydroxide solution (10 ml) and the reaction mixture was stirred at room temperature for 12 h. The solution was acidified to pH 7 with 0.5 M sulfuric acid, and the precipitate was collected by filtration to give **17** (20 mg, 0.049 mmol, 50%). mp 213—215 °C (dec.); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 1.78—1.85 (m, 1H, β-H), 1.95—2.06 (m, 1H, β-H), 2.64 (t, 2H, J=7.5 Hz, γ-H), 2.81 (dd, 1H, J=13.7, 6.6 Hz, 5′-H), 2.95 (dd, 1H, J=13.5, 4.5 Hz, 5′-H), 3.28 (dd, 1H, J=6.5, 5.4 Hz, α-H), 3.87 (dd, 1H, J=10.7, 5.9 Hz), 4.13 (t, 1H, J=6.3 Hz), 4.54 (t, 1H, J=5.6 Hz), 5.45 (br s, 2H, 2×OH), 6.07 (d, 1H, J=4.9 Hz, 1′-H), 7.29—7.34 (m, 1H, Ph), 7.54—7.57 (m, 1H, Ph), 7.70—7.75 (m, 1H, Ph), 8.00—8.03 (m, 1H, Ph); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 28.9, 31.7, 34.0, 53.4, 69.7, 72.1, 82.7, 90.8, 116.3, 116.6, 123.7, 128.0, 135.4, 140.4, 150.3, 161.9, 170.0; UV λ_{max} nm (ε): 306 (3433, pH=1), 305 (3830, MeOH/H_2O), 307 (2542, pH=11); MS (ESI, formic acid) *m/z* 338 (45), 412 (100) (M^++1); HR-MS (ESI, formic acid) Calcd for $C_{17}H_{22}N_3O_7S$ (M+1): 412.1178, Found: 412.1172.

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