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TETRAHEDRON

Conversion of Uridine into 2'-*O*-(2-Methoxyethyl)-uridine and 2'-*O*-(2-Methoxyethyl)cytidine

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Abstract: Reaction between aluminium 2-methoxyethoxide and 2,2'-anhydro-1-β-D-arabinofuranosyluracil **6** gives 2'-*O*-(2-methoxyethyl)uridine **9** in high yield. Compound **9** is converted into 2'-*O*-(2-methoxyethyl)cytidine **11** in good yield. © 1999 Elsevier Science Ltd. All rights reserved.

The possibility that synthetic oligonucleotides might be effective inhibitors of gene expression and be used as chemotherapeutic agents[1] has stimulated much research work in recent years. In order to avoid their degradation by cellular nucleases, it is essential that such oligonucleotides should be modified.[2] Modifications can be made to the internucleotide linkages, the base residues and the sugar residues. A large number of oligonucleotide analogues in which the internucleotide linkages have been modified, especially as phosphorothioates with non-bridging sulfur atoms,[3] have been described. Several of these phosphorothioate analogues are promising drug candidates[4] that are now undergoing clinical trials, and one of them has already been approved by the U.S. Food and Drug Administration. However, phosphorothioates have some disadvantages[5] in that they do not display optimal RNA-binding properties and have a tendency to bind to proteins in a non-specific manner. Possible base modifications are clearly limited as they must not lead to a significant decrease in hybridisation properties. Recently, considerable interest has been directed towards the modification of the sugar residues. One particular type of modification involves the introduction of 2'-α-alkoxy groups[6] (as in 2'-*O*-alkyl-oligoribonucleotides **1**). While, in general, small alkoxy groups (such as methoxy, as in **1a**) promote better hybridisation properties with complementary ribonucleic acids (RNA), nuclease resistance tends to increase with an increase in the size of the alkoxy group.[2] 2-Methoxyethoxy (as in **1b**) has emerged[2,5,7] as an alkoxy group that confers both good hybridisation properties and high nuclease resistance. It therefore seems likely[5] that 2'-*O*-(2-methoxyethyl)-ribonucleosides **2** will be incorporated into a second generation of potential oligonucleotide chemotherapeutic agents. For this reason, the development of convenient methods for the preparation of 2'-*O*-(2-methoxyethyl)-ribonucleosides **2** has become a matter of much importance.

The preparation of 2'-*O*-(2-methoxyethyl)-ribonucleosides **2**, starting from D-ribose, has previously been described.[7] These preparations involved the use of protecting groups and required a relatively large number of steps. For example, 2'-*O*-(2-methoxyethyl)-5-methyluridine **3** was prepared[7] (Scheme 1a) from D-ribose in 10 steps and in *ca.* 33% overall yield. A later report by McGee and Zhai[8] revealed a much more convenient procedure for the preparation of 2'-*O*-alkyl derivatives of the main pyrimidine ribonucleosides. Thus, when 5'-*O*-(4,4'-dimethoxytrityl)-2,2'-anhydro-1-β-D-arabinofuranosyluracil **4** was heated with magnesium methoxide in *N*,*N*-dimethylformamide (DMF) at 100°C (Scheme 1b), 5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-methyluridine **5**; R = Me was obtained in 94% yield. Somewhat lower yields (54, 62 and 42%, respectively) of the corresponding 2'-*O*-ethyl-,

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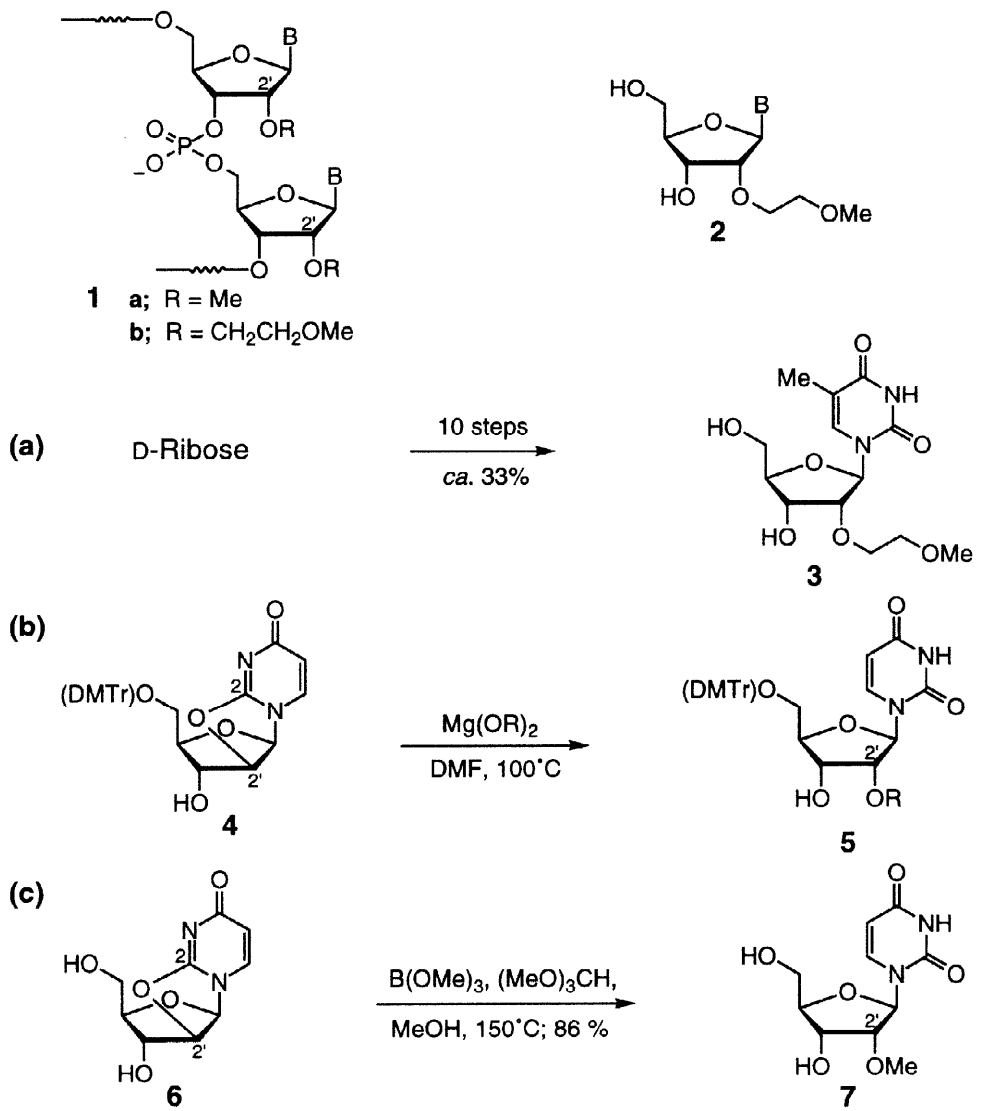
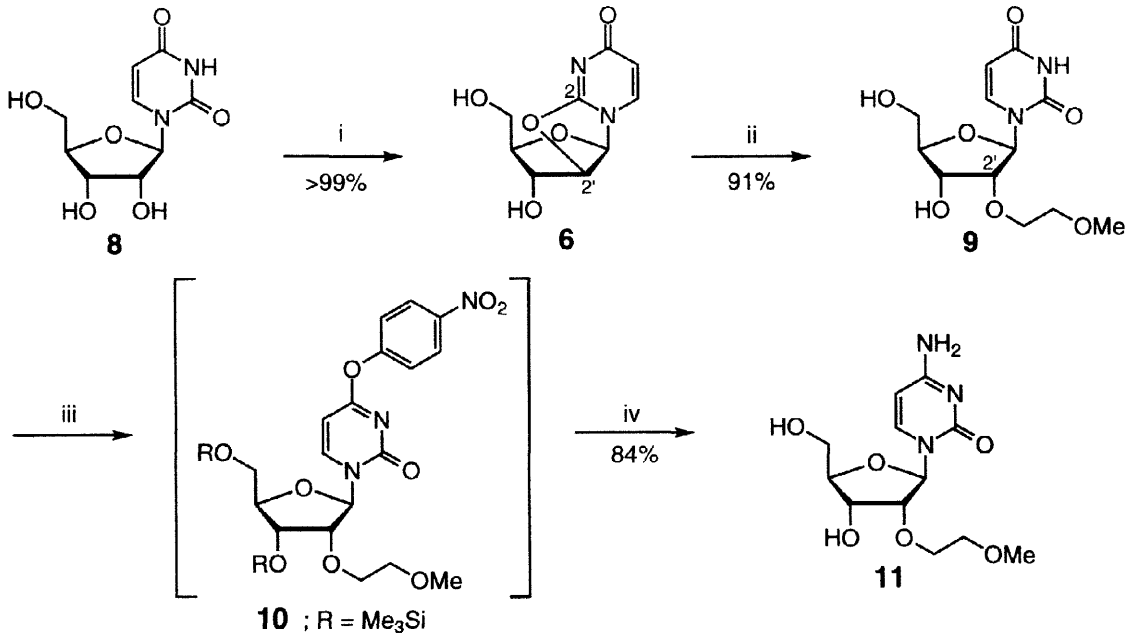


Scheme 1

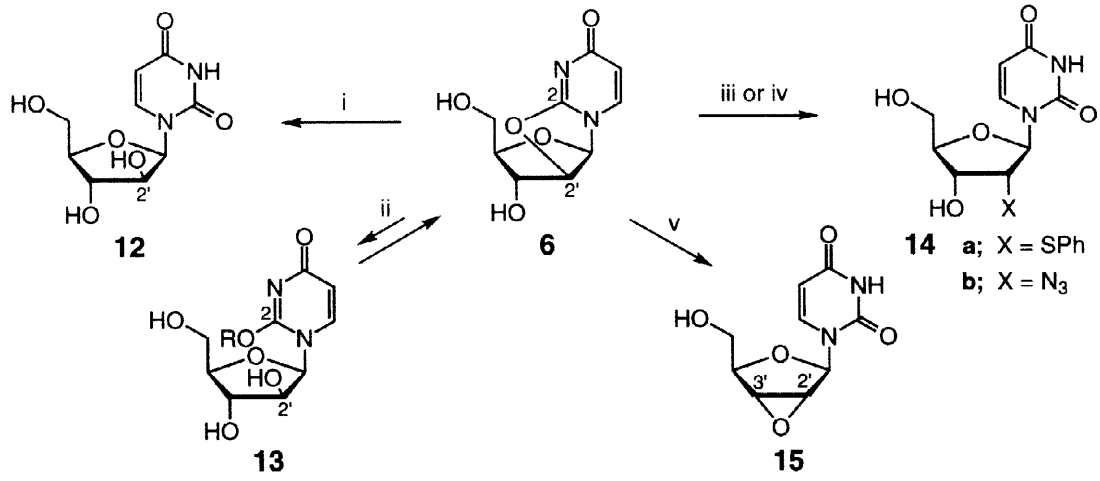
2'-*O*-propyl- and 2'-*O*-allyl-uridine derivatives (**5**; R = Et, Pr and allyl, respectively) were obtained in the reactions between the same substrate **4** and the appropriate magnesium alkoxides. It was also reported that magnesium alkoxides could be replaced by calcium alkoxides. McGee and Zhai[8] alluded to the possible mechanism of this reaction by suggesting that the free 3'-hydroxy function assisted the 'intramolecular delivery of a divalent metal alkoxide' leading to the regiospecific opening of the anhydronucleoside derivative at the 2'-position. More recently, Ross *et al.* reported[9] that when *unprotected* 2,2'-anhydro-1-β-D-arabinofuranosyluracil **6** was heated with a twofold excess of trimethyl borate and a stoichiometric quantity of trimethyl orthoformate in methanol at 150°C, under pressure, for 42 h, 2'-*O*-methyluridine **7** was obtained (Scheme 1c) in 86% isolated yield. 2'-*O*-Methyl-5-methyluridine was similarly prepared from 2,2'-anhydro-5-methyl-(1-β-D-arabinofuranosyluracil) by the borate ester procedure[9] and, although no experimental details were provided, the preparation of 2'-*O*-methylcytidine was also reported. Ross *et al.* stated[9] that the yield of 2'-*O*-alkyl-uridine decreased with increasing alcohol 'size'. Despite the fact that the 5'-hydroxy function of their substrate **6** was unprotected and boron is trivalent, Ross *et al.* suggested[9] that the participation of the neighbouring 3'-hydroxy function might again be involved.



Scheme 2 *Reagents and conditions*: i, $(PhO)_2CO$ (1.1 mol equiv.), $NaHCO_3$ (0.05 mol equiv.), DMA, 100 °C, 5h; ii, $Al(OCH_2CH_2OMe)_3$ (*ca.* 1.0 M, 3.0 mol equiv.), reflux, 48 h; iii, (a), Me_3SiCl, 1-methylpyrrolidine, MeCN, rt, 1 h, (b), $(CF_3CO)_2O$, 0 °C, 30 min, (c), 4-nitrophenol, 0 °C, 3 h; iv, conc. aq. NH_3 (d 0.88)-dioxane (1 : 5 v/v), 55 °C, 24 h.

We now report that when 2,2'-anhydro-1-β-D-arabinofuranosyluracil[10-12] **6** was heated, under reflux and under anhydrous conditions, with a threefold excess of *ca.* 1.0 M aluminium 2-methoxyethoxide in dry 2-methoxyethanol for 48 h *at atmospheric pressure*, 2'-*O*-(2-methoxyethyl)uridine **9** was obtained (Scheme 2) as virtually the sole nucleoside product, and was isolated in 91% overall yield for the two steps starting from uridine **8**. 2,2'-Anhydro-1-β-D-arabinofuranosyluracil **6** was obtained in quantitative yield by heating uridine **8** with a 5% excess of diphenyl carbonate and a catalytic amount of sodium hydrogen carbonate in *N*,*N*-dimethylacetamide (DMA) at 100°C. A relatively small quantity of DMA (*ca.* 0.8 mL / g of uridine) was used, and the anhydro-nucleoside **6**, contaminated with salts, was isolated by precipitation and used without further purification. We have found that DMA is a more satisfactory solvent than DMF[10,12] in the preparation of 2,2'-anhydro-1-β-D-arabinofuranosyluracil **6**. Aluminium 2-methoxyethoxide was prepared by heating aluminium foil and 2-methoxyethanol, under reflux, for *ca.* 1 h. 2'-*O*-(2-Methoxyethyl)uridine **9** was converted into the corresponding cytidine derivative **11** by the 4-nitrophenylation[12,13] (Scheme 2, steps iii and iv) rather than by the more commonly used triazolation procedure.[14] Following trimethylsilylation in acetonitrile, the resulting 2'-*O*-(2-methoxyethyl)uridine derivative was allowed to react first with trifluoroacetic anhydride[15] and 1-methylpyrrolidine,[16] and then with 4-nitrophenol. The putative intermediate 4-*O*-(4-nitrophenyl) derivative **10** was was treated directly with ammonia in aqueous dioxane to give 2'-*O*-(2-methoxyethyl)cytidine **11**, which was isolated in 84% overall yield.

It would seem likely that the conversion of 2,2'-anhydro-1-β-D-arabinofuranosyluracil **6** into 2'-*O*-(2-methoxyethyl)uridine **9** could be speeded up appreciably by carrying out the reaction at a higher temperature in a sealed vessel. It would further seem probable that 5-methyluridine could be converted by the same two-step process (Scheme 2, steps i and ii) into 2'-*O*-(2-methoxyethyl)-5-methyluridine[7] **3** in high yield, and that the latter compound **3** could be converted into the corresponding 5-methylcytidine derivative[7] by the nitrophenylation-ammonolysis procedure[12] (Scheme 2, steps iii and iv).



Scheme 3 *Reagents*: i, Et_3N, H_2O; ii, NaOR, ROH; iii, PhSH, Et_3N, MeCN; iv, LiN_3, $PO(NMe_2)_3$; v, NaH or $KOBu^t$, DMSO.

The chemistry of the action of nucleophiles on 2,2'-anhydro-1-β-D-arabinofuranosyluracil **6** (Scheme 3) is complicated. Hydroxide ion, which is a 'hard' nucleophile, attacks irreversibly at *C*-2 to give 1-β-D-arabinofuranosyluracil[14] **12**. Alcoholic solutions of alkali metal alkoxides would also be expected to attack at *C*-2 to give 2-*O*-alkyl derivatives **13** of 1-β-D-arabinofuranosyluracil. However, such reactions would be expected to be reversible and to favour the anhydronucleoside **6**. Thiophenate[17] and azide[11] ions are examples of 'soft' nucleophiles that are known to attack 2,2'-anhydro-1-β-D-arabinofuranosyluracil **6** at *C*-2' to give compounds **14a** and **14b**, respectively. Under strongly basic conditions (e.g. in the presence of an excess of sodium hydride[12,18] or potassium *tert*-butoxide[12] in DMSO solution) the 2,2'-anhydronucleoside **6** is converted into the isomeric 2',3'-epoxide[12,18] **15**, which is then susceptible to nucleophilic attack at the β - face of *C*-3'. In the present study, aluminium 2-methoxyethoxide appears to be behaving effectively as a 'soft' nucleophile. Attack at *C*-2 (Scheme 3, reaction ii) is reversible, and would not therefore be expected to lead to an isolable product. Furthermore, aluminium 2-methoxyethoxide is not sufficiently basic to promote the conversion of the substrate **6** into the 2',3'-epoxide[12,18] **15**. Therefore the only possible irreversible reaction is attack at *C*-2'. It is reasonable to conclude that it is unnecessary to invoke the participation of the neighbouring 3'-hydroxy function in the present study (*i.e.* in Scheme 2, reaction ii), and also perhaps in the reactions between 2,2'-anhydro-1-β-D-arabinofuranosyluracil **6** and magnesium alkoxides[8] and trialkyl borates.[9]

EXPERIMENTAL

Mps are uncorrected. ^1H and ^{13}C NMR spectra were measured at 360.1 and 90.6 MHz respectively, with a Bruker AM 360 spectrometer; tetramethylsilane was used as an internal standard. TLC was carried out with Merck silica gel 60 F_{254} pre-coated plates (Art 5715), which were developed in solvent system A [$CHCl_3$-MeOH (85 : 15 v/v)]. Short column chromatography was carried out on silica gel (Merck Art 7729). Acetonitrile and 1-methylpyrrolidine were dried by heating, under reflux, with calcium hydride and were then distilled. *N,N*-Dimethylacetamide (DMA) was dried by distillation over calcium hydride under reduced pressure. 2-Methoxyethanol was dried by heating with aluminium foil (1 g / 250 mL), under reflux, and was then distilled. Diethyl ether was dried over sodium wire.

2,2'-Anhydro-1-β-D-arabinofuranosyluracil 6

Uridine (12.21 g, 50 mmol), diphenyl carbonate (11.79 g, 55 mmol), sodium hydrogen carbonate (0.21 g, 2.5 mmol) and dry DMA (10 mL) were heated together, with stirring, at 100°C. After 5 h, the products were cooled to rt and diethyl ether (100 mL) was added with stirring. After 2 h, the colourless precipitate (11.70 g) was collected by filtration and washed with ether (2 x 50 mL). The sole nucleoside constituent [R_f 0.12 (system A); δ_H [$(CD_3)_2SO$] 3.17 (1 H, m), 3.27 (1 H, m), 4.06 (1 H, m), 4.37 (1 H, s), 4.98 (1 H, t, *J* 5.0), 5.19 (1 H, d, *J* 5.7), 5.83 (1 H, d, *J* 7.5), 5.89 (1 H, br), 6.30 (1 H, d, *J* 5.7), 7.83 (1 H, d, *J* 7.4). δ_C [$(CD_3)_2SO$] 60.9, 74.8, 88.8, 89.3, 90.1, 108.7, 137.0, 159.9, 171.4] of the precipitated material was identified as 2,2'-anhydro-1-β-D-arabinofuranosyluracil **6** (calculated quantitative yield, 11.31 g) by comparison with authentic material.[11]

2'-*O*-(2-Methoxyethyl)uridine 9

Aluminium foil (3.64 g, 0.135 mol) and dry 2-methoxyethanol (135 mL) were heated, under reflux, for *ca.* 1 h until all of the aluminium had been consumed. Crude (see above) 2,2'-anhydro-1-β-D-arabinofuranosyluracil **6** (10.18 g, *ca.* 43.5 mmol) was added and the reactants were heated, under reflux, for 48 h. Absolute ethanol (200 mL), followed by water (7.3 mL, 0.405 mol) and Celite were added to the cooled products. The resulting mixture was heated, under reflux, for 10 min and was then filtered. The residue was washed with ethanol (3 x 100 mL). The combined filtrate and washings were evaporated under reduced pressure to give a pale yellow solid. The material was purified by short column chromatography on silica gel (70 g): the appropriate fractions, which were eluted with CH_2Cl_2 - MeOH (90 : 10 v/v), were evaporated under reduced pressure to give the *title compound* **9** as a colourless solid (12.05 g, *ca.* 91%) (Found, in material recrystallized from ethyl acetate : C, 47.69; H, 5.96; N, 9.25. $C_{12}H_{18}N_2O_7$ requires : C, 47.68; H, 6.00; N, 9.27%), m.p. 124-125°C; R_f 0.45 (system A); λ_{max} (H_2O)/nm 261 (ε 9 800); λ_{min}/nm 230 (ε 2 300); δ_H [$(CD_3)_2SO$] 3.22 (3 H, s), 3.44 (2 H, m), 3.55 (1 H, m), 3.60 - 3.71 (3 H, m), 3.85 (1 H, m), 3.95 (1 H, m), 4.09 (1 H, m), 5.06 (1 H, d, *J* 5.7), 5.15 (1 h, t, *J* 5.0), 5.65 (1 H, d, *J* 8.1), 5.84 (1 H, d, *J* 5.1), 7.93 (1 H, d, *J* 8.1), 11.36 (1 H, br s); δ_C [$(CD_3)_2SO$] 58.0, 60.4, 68.3, 68.9, 71.1, 81.3, 84.9, 86.0, 101.7, 140.4, 150.5, 163.0.

2'-*O*-(2-Methoxyethyl)cytidine 11

2'-*O*-(2-Methoxyethyl)uridine **9** (6.05 g, 20.0 mmol), 1-methylpyrrolidine (20 mL, 0.192 mol), chlorotrimethylsilane (7.6 mL, 59.9 mmol) and dry acetonitrile (100 mL) were stirred together at rt. After 1 h, the reaction mixture was cooled to 0°C (ice-water bath) and trifluoroacetic anhydride (7.1 mL, 50.3 mmol) was added dropwise over 5 min. After a further period of 30 min at 0°C, 4-nitrophenol (8.35 g, 60 mmol) was added to the stirred reactants which were maintained at 0°C. After 3 h, the products were poured into saturated aqueous sodium hydrogen carbonate (200 ml), and the resulting mixture was extracted with dichloromethane (3 x 100 mL). The combined organic layers were dried ($MgSO_4$), and evaporated under reduced pressure. Concentrated aqueous ammonia (*d* 0.88, 20 mL) was added to a stirred solution of the residue in dioxane (100 mL), contained in a sealed flask that was then heated at 55°C for 24 h. The resulting yellow solution was concentrated under reduced pressure and the residue was evaporated with absolute ethanol (3 x 50 mL). The products were fractionated by short colum chromatography on silica gel: the appropriate fractions, which were eluted with CH_2Cl_2 - MeOH - Et_3N (93 : 7 : 0.5 to 90 : 10 : 0.5 v/v) were evaporated under reduced pressure to give the *title compound* **11** as an off-white solid (5.07 g, 84%) (Found, in material recrystallized from MeOH- Et_2O : C, 47.79; H, 6.24; N, 13.78. $C_{12}H_{19}N_3O_6$ requires : C, 47.84; H, 6.36; N, 13.96%), m.p. 154-156°C; R_f 0.10 (system A); δ_H [$(CD_3)_2SO$] 3.23 (3 H, s), 3.45 (2 H, t, *J* 4.8), 3.55 (1 H, m), 3.64 - 3.77 (3 H, m), 3.81 (2 H, m), 4.04 (1 H, m), 4.96 (1 H, d, *J* 6.1),

5.12 (1 H, t, *J* 5.1), 5.72 (1 H, d, *J* 7.4), 5.83 (1 H, d, *J* 3.9), 7.22 (2 H, br s), 7.90 (1 H, d, *J* 7.4); δ_C [$(CD_3)_2SO$] 58.0, 60.1, 68.0, 68.8, 71.1, 81.9, 84.0, 87.2, 93.8, 141.1, 155.1, 165.5.

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