Synthesis of 4-(1,2,4-Triazol-1-yl)pyrimidin-2(1*H*)-one Ribonucleotide and Its Application in Synthesis of Oligoribonucleotides

Wing L. Sung

Division of Biological Sciences, National Research Council of Canada,[1] Ottawa, Canada K1A OR6

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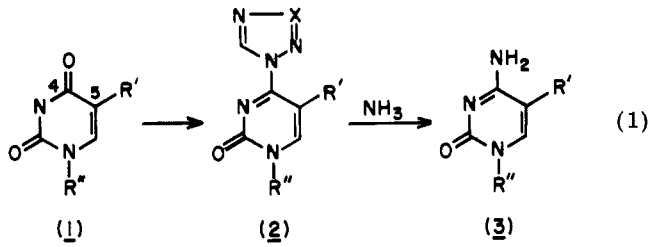
5′-*O*-(Dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)uridine (**21**) was converted to 4-(1,2,4-triazol-1-yl)-1-[5′-*O*-(dimethoxytrityl)-3′-*O*-((*p*-chlorophenoxy)(2-cyanoethoxy)phosphoryl)-2′-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]pyrimidin-2(1*H*)-one (**20**), which was then condensed with other ribonucleotides via the modified phosphotriester method to give oligonucleotides. Subsequent deprotection of the latter under different conditions converted the 4-(1,2,4-triazol-1-yl)pyrimidin-2-one moiety to cytosine (ammonia in dioxane), uracil (N^1,N^1,N^3,N^3-tetramethylguanidinium *syn*-2-pyridinealdoximate), or N^4,N^4-dimethylcytosine (dimethylamine). An efficient synthesis of 4-methoxypyrimidin-2-one riboside was also accomplished via transformation of the 4-triazolyl moiety by methanol in the presence of ammonia.

In a study of side reactions during oligonucleotide synthesis, Reese and Ubasawa observed conversion of the uracil moiety (1, R′ = H) to 4-tetrazolylpyrimidinone (**2**, R′ = H, X = N) by mesitylenesulfonyl tetrazolide[2] (eq 1).



(1)

(1) (2) (3)

Simultaneously, study of phosphorylation of nucleosides in our laboratory indicated that *p*-chlorophenyl phosphorodichloridate and 1,2,4-triazole are capable of similar modification. Thymidine (1, R′ = CH_3), which resists modification by mesitylenesulfonyl tetrazolide,[2] has been successfully converted the to 4-(1,2,4-triazolyl)pyrimidinone derivative (**2**, R′ = CH_3, X = CH). Subsequent ammonia treatment yields 5-methyldeoxycytidine (**3**, R′ = CH_3).[3] By use of the same general approach, biologically important oligonucleotides containing 5-methylcytosine have been synthesized.[4]

Application of this base-modification (1 → **2** → **3**) in oligonucleotide synthesis has now been extended to the ribose series. It is conceivable that the same combination of *p*-chlorophenyl phosphorodichloridate and 1,2,4-triazole, as both phosphorylating and base-modifying agents, can convert 2′,5′-protected uridine (**4**) or its nucleotide (**5**) to 4-(1,2,4-triazolyl)pyrimidinone nucleotide **6** (Scheme I).

Coupling between **6** and other common nucleotides via the phosphorotriester approach would give a fully protected oligonucleotide (**7**). Under different deblocking conditions, **7** can be deprotected to phosphorodiesters (**8**–**10**), with concomitant conversion of the 4-triazolylpyrimidinone moiety to cytosine (ammonia), uracil (oximate ion), or N^4-alkylcytosine (alkylamine).[2-4]

This approach of using 4-triazolylpyrimidinone as a precursor for specific pyrimidines has now been demonstrated in the synthesis of three heptanucleotides, 5′-OH-AUUUA*UC*-3′,2′-OH, 5′-OH-AUUUACC-3′,2′-OH, and 5′-OH-ACUACGA-3′,2′-OH, identical with the two closely related intervening sequences AUUUA*UC*ACUACGA and AUUUACCACUACGA in tyrosine transfer RNA from three unlinked genetic loci in yeast.[5]

The significance of this approach is two-fold: (i) in the synthesis of ACUACGA, modified nucleotide **6** as a substitute for cytidine (C) will couple with the other three nucleotides, (A, G, U) with subsequent deprotection by ammonia, rendering the generally laborious preparation of the N^4,5′,2′-protected cytidine 3′-phosphorotriester unnecessary; (ii) in the synthesis of AUUUA*UC* and AUUUACC, **6** as a "variable pyrimidine precursor" for both *U* and *C* will couple with the other common nucleotides (A, C, U) to yield a single fully protected oligonucleotide. Upon different deblocking procedures (ammonia, oximate, or alkylamine),[6,7] a family of closely related oligonucleotides (**8**–**10**) can be obtained, which, in the past, have had to be synthesized individually according to their specific sequences.[8,9]

Exploratory work on this modification in uridine is shown in Scheme II. 5′-*O*-(Dimethoxytrityl)-2′,3′-*O*-bis(*tert*-butyldimethylsilyl)uridine (**11**) was treated with *p*-chlorophenyl phosphorodichloridate and 1,2,4-triazole to yield the 4-triazolylpyrimidinone derivative **12** (312 nm, two singlets at δ 9.08 and 7.91).[2-4]

Substitution of the 4-triazolyl group of **12** with ammonia in dioxane yielded the cytidine derivative **13** (275 nm), identical with a sample prepared from 5′-*O*-(dimethoxytrityl)-N^4-benzoyl-2′,3′-bis(*tert*-butyldimethylsilyl)cytidine via debenzoylation (ammonia).[10] Treatment of **12** with methylamine and dimethylamine instantaneously gave the N^4-methyl and N^4,N^4-dimethyl analogues (**14** and **15**) of cytidine.[11]

Reactivity of the 4-triazolyl group of **12** prompted us to define its limitations, so that an appropriate strategy could be designed for subsequent synthesis. **12** was further treated with various amines which have been used extensively in synthesis of oligoribonucleotides. In hydrazine, **12** was converted readily to 4-hydrazinylpyrimidinone derivative **16**.[11] Therefore, hydrazinolysis, which has been used for removal of various protecting groups,[8,12-14] is not

(1) NRCC Publication No. 20516.
(2) C. B. Reese and A. Ubasawa, *Tetrahedron Lett.*, 2265 (1980).
(3) W. L. Sung, *J. Chem. Soc., Chem. Commun.*, 1089 (1981).
(4) W. L. Sung, *Nucleic Acids Res.*, **9**, 6139 (1981).
(5) H. M. Goodman, M. V. Olson, and B. D. Hall, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5453 (1977).
(6) C. B. Reese and L. Zard, *Nucleic Acids Res.*, **9**, 4611 (1981).
(7) S. A. Narang, R. Brousseau, H. M. Hsiung, and J. J. Michniewicz, *Methods Enzymol.* **65**, 610–619 (1980).
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(9) J. H. van Boom, P. M. J. Burgers, R. Crea, G. van der Marel, and G. Wille, *Nucleic Acids Res.*, **4**, 747 (1977).
(10) K. K. Ogilvie, A. L. Schifman, and C. L. Penny, *Can. J. Chem.*, **57**, 2230 (1979).
(11) J. J. Fox, D. van Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, *J. Am. Chem. Soc.*, **81**, 178 (1959).
(12) K. K. Ogilvie and M. J. Nemer, *Can. J. Chem.*, **58**, 1389 (1980).

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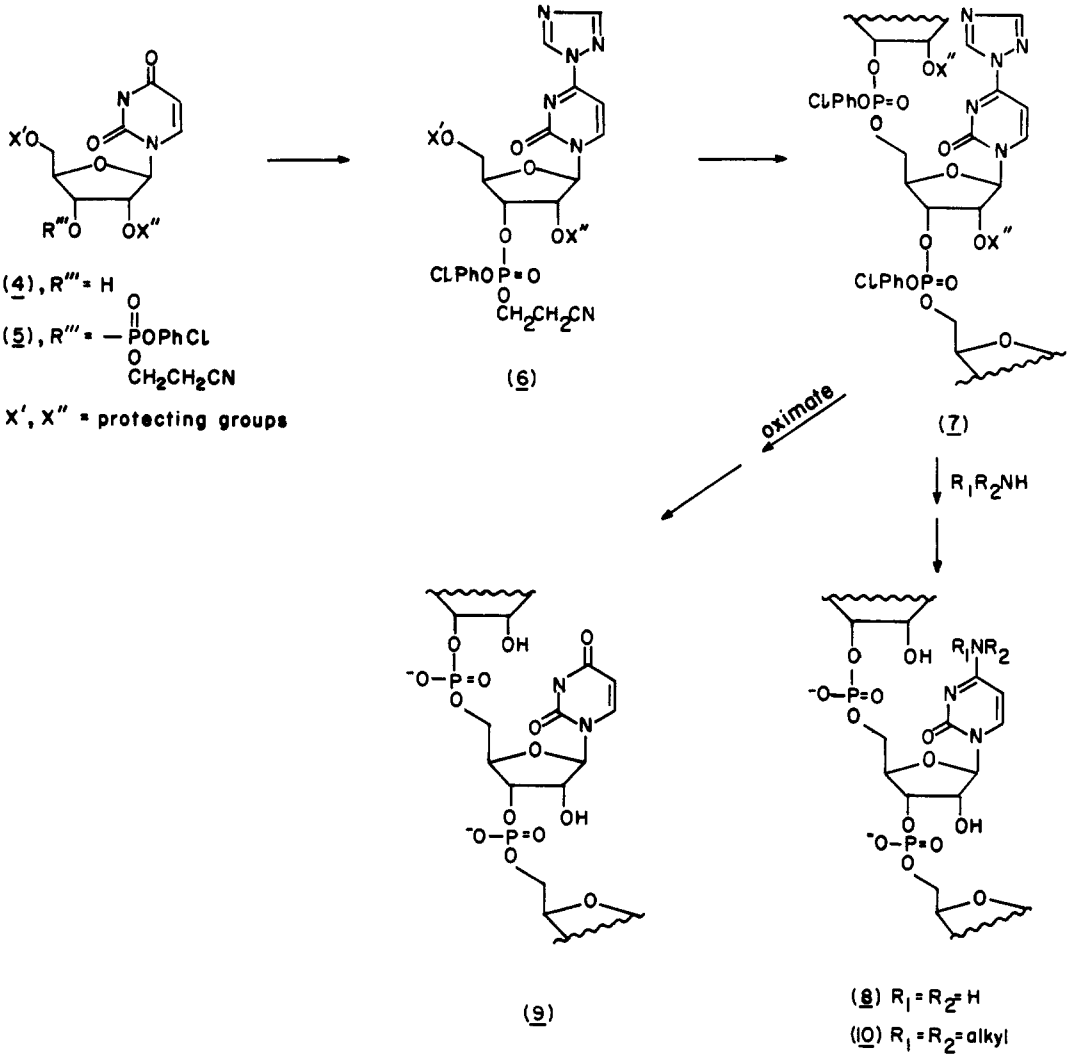
Scheme I



Scheme II

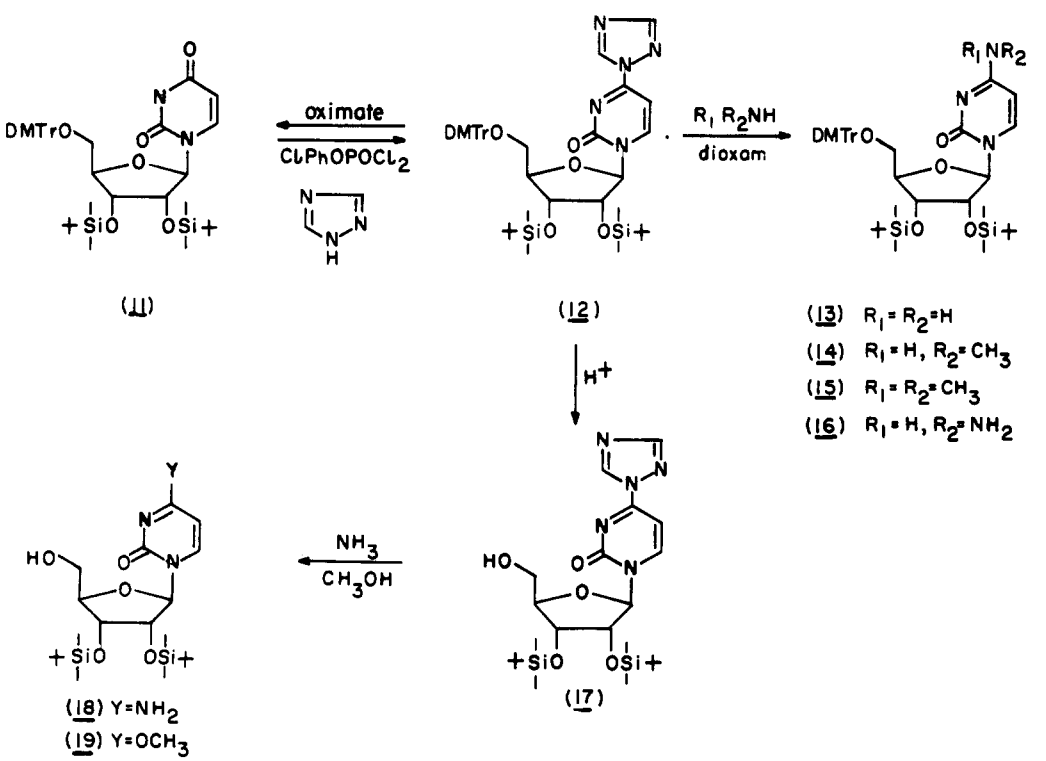


Table I. Synthesis of the Fully Protected Heptamers AUUUA*M*C and A*M*UA*M*GA

5′-protected[a] component (mmol)	3′-hydroxyl[b] component (mmol)	coupling agent[d,e] (mmol)	product (% yield)
	AUUUA*M*C		
[(MeO)$_2$Tr]*M*-ClPh[c] (0.42)	bzC-(OSi)$_2$ (0.38)	0.51	[(MeO)$_2$Tr]*M*±bzC -(OSi)$_2$ (69)
[(MeO)$_2$Tr]U±bzA -ClPh (0.18)	*M*±bzC-(OSi)$_2$ (0.12)	0.26	[(MeO)$_2$Tr]U±bzA±*M* ±bzC-(OSi)$_2$ (55)
[(MeO)$_2$Tr]bzA±U ±U-ClPh (0.12)	U±bzA±*M*±bzC-(OSi)$_2$ (0.06)	0.24	[(MeO)$_2$Tr]bzA±U±U±U ±bzA±*M*±bzC-(OSi)$_2$ (49)
	A*M*UA*M*GA		
[(MeO)$_2$Tr]bzA- ClPh (0.56)	*M*±CE (0.48)	0.70	[(MeO)$_2$Tr]bzA±*M* ±CE (71)
[(MeO)$_2$Tr]bzA±*M* -ClPh (0.14)	U±CE (0.18)	0.21	[(MeO)$_2$Tr]bzA±*M* ±U±CE (55)
[(MeO)$_2$Tr]bzA±*M* -ClPh (0.18)	IsoG±bzA-(OSi)$_2$ (0.21)	0.28	[(MeO)$_2$Tr]bzA±*M* ±IsoG±bzA-(OSi)$_2$ (48)
[(MeO)$_2$Tr]bzA±*M* ±U-ClPh (0.07)	bzA±*M*±IsoG±bzA-(OSi)$_2$ (0.06)	0.12	[(MeO)$_2$Tr]bzA±*M*±U±bzA±*M*±IsoG±bzA -(OSi)$_2$ (41)

[a] Abbreviations are as suggested by IUPAC-IUB [*Biochemistry*, 9, 4022 (1970)]. A phosphodiester linkage is represented by a hyphen and a phosphorotriester linkage is represented by a (±) symbol. Each internucleotide phosphate is protected by a *p*-chlorophenyl (ClPh) group, and each 2′-hydroxyl of each nucleoside is protected by a *tert*-butyldimethylsilyl group. [b] The 2′- and 3′-hydroxyl groups of the 3′-terminal residue are protected by two *tert*-butyldimethylsilyl groups (OSi) or a 3′-O(*p*-chlorophenoxy)(β-cyanoethoxy)phosphoryltriester. Exocyclic amines of A, C, and G are protected by a benzoyl (bz) or an isobutyryl (Iso) group. [c] *M* is 4-(1,2,4-triazol-1-yl)-1-[2′-*O*-*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]pyrimidin-2(1*H*)-one 3′-phosphate. [d] Mesitylenesulfonyl tetrazolide. [e] The reaction time was 15 min in all cases.

applicable in our present studies. However, **12** remained stable in both diisopropylamine and triethylamine (5 days), which have been used for generation of 3′-phosphorodiester functional group in some syntheses.[7]

The dimethoxytrityl group of **12** was easily removed by 2% benzenesulfonic acid to yield **17** quantitatively, therefore indicating this group to be appropriate for protecting the 5′-hydroxyl function.[7]

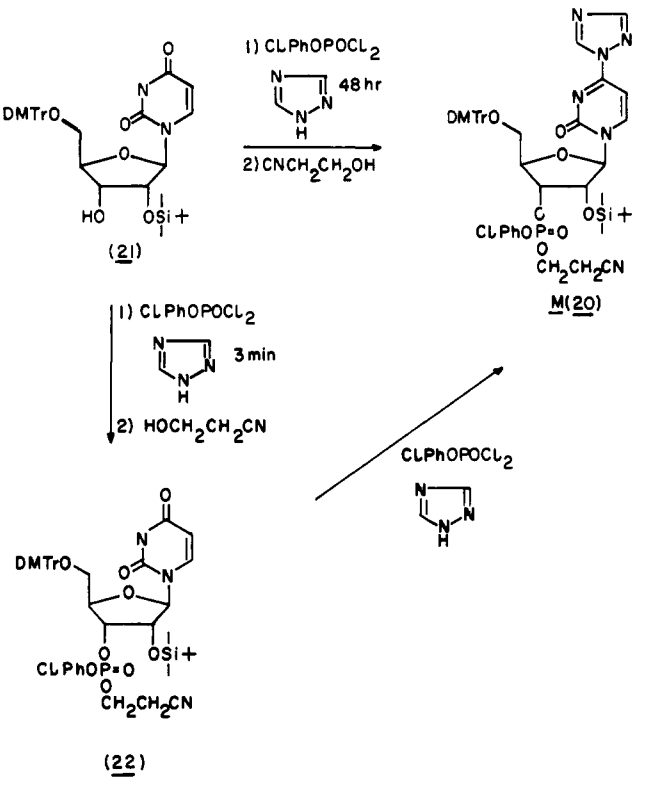
When **17** was treated with aqueous ammonia in methanol,[15] instead of dioxane, only a small amount of the expected cytidine derivative (**18**) was obtained.[10] Most of **17** was converted to the 4-methoxypyrimidinone derivative **19**.[16] Although other reports indicated that 4-methoxypyrimidinone could ultimately be converted to cytosine after prolonged treatment, the drastic conditions (100 °C in a sealed tube) required and the instability of this base moiety in acidic medium render this transformation route unfavorable in subsequent synthesis.[16] Nevertheless, our results provided a new procedure for the synthesis of 4-methoxypyrimidinone riboside, which had been prepared under relatively vigorous conditions.[16]

In a solution of N^1,N^1,N^3,N^3-tetramethylguanidinium *syn*-2-pyridinealdoximate, **12** was readily converted back to uridine derivative **11**.[2]

Since the methodology of using ribonucleotides (A, C, G, U) in the form of a 5′-*O*-(dimethoxytrityl)-2′-(*tert*-butyldimethylsilyl)-3′-*O*-[(*p*-chlorophenoxy)(2-cyanoethoxy)phosphoryl] triester for oligonucleotide synthesis has been developed,[17,18] it was adopted for the present synthesis.

For preparing the 4-triazolylpyridin-2-one nucleotide *M* (**20**), 5′-*O*-(dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)uridine (**21**) was treated with an excessive amount of 1,2,4-triazole and *p*-chlorophenyl phosphorodichloridate in pyridine for 48 h to yield the base-modified phosphorodiester (Scheme III). Addition of β-cyanoethyl yielded

Scheme III



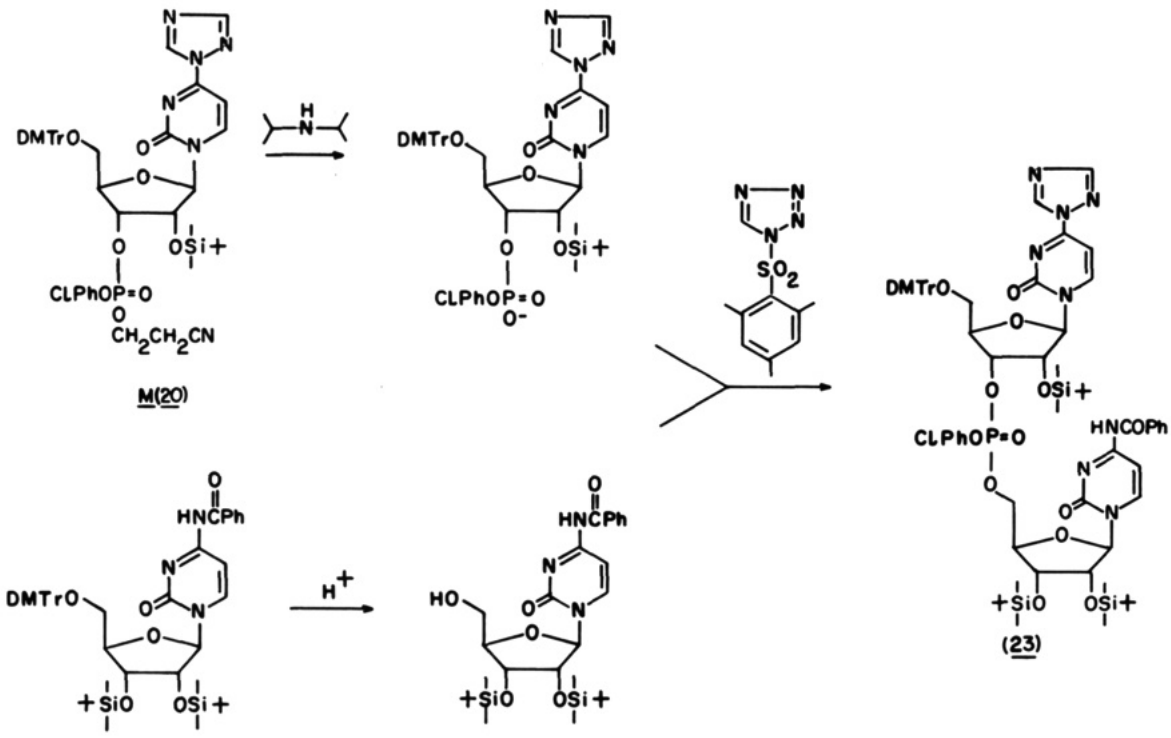
the modified nucleotide *M* (**20**).

In contrast, when the interval for phosphorodiester formation was shortened to 3 min, the same procedure yielded only unmodified uridine nucleotide **22**. This result indicates that the phosphorylation of **21** was completed almost instantaneously, thus eliminating any possibility of migration of the 2′-silyl group in the synthesis of *M* (**20**).[10] By the same modifying procedure, uridine nucleotide **22** was converted to *M* (**20**).

The two 4-triazolylpyrimidinone-containing heptamers AUUUA*M*C and A*M*UA*M*GA were synthesized by coupling between protected nucleotides (A, C, G, U, *M*) via a three-step approach (Scheme IV):[7] (i) decyanoethylation

(13) R. L. Letsinger, M. H. Caruthers, P. S. Miller, and K. K. Ogilvie, *J. Am. Chem. Soc.*, **89**, 7146 (1967).
(14) R. J. Gregoire and T. Neilson, *Can. J. Chem.*, **56**, 487 (1978).
(15) E. S. Werstiuk and T. Neilson, *Can. J. Chem.*, **50**, 1283 (1972).
(16) M. J. Robin and S. R. Naik, *Biochemistry*, **10**, 3591 (1971).
(17) W. L. Sung and S. A. Narang, *Can. J. Chem.*, **60**, 111 (1982).
(18) K. K. Ogilvie and R. T. Pon, *Nucleic Acids Res.*, **8**, 2105 (1980).

Scheme IV



of one nucleotide block to give 3′-phosphorodiester function by diisopropylamine; (ii) detritylation of another block by 2% benzenesulfonic acid to expose the 5′-hydroxyl group; (iii) coupling of the two treated units in the presence of 1-(mesitylene-2-sulfonyl)tetrazole. The strategy and other data of the synthesis are presented in Table I.

Both fully protected heptamers AUUUA*MC* and A*M*-UA*MG*A were treated with ammonia for the removal of the *p*-chlorophenyl groups at the phosphate linkage and various acyl groups at the exocyclic amines. The 4-triazolylpyrimidinones of the heptamers were simultaneously converted to cytosine by ammonia.[2] The dimethoxytrityl group at the 5′ end was removed by acetic acid–water (4:1).[19] Finally, desilylation by tetrabutylammonium fluoride gave the unprotected phosphorodiesters 5′-OH-AUUUA*CC*-2′,3′-OH and 5′-OH-A*CUAC*GA-2′,3′-OH.[10]

Via a different deblocking procedure, fully protected heptamer AUUUA*MC* was first treated with N^1,N^1,N^3,N^3-tetramethylguanidinium *syn*-2-pyridinealdoximate,[6] instead of ammonia, for removal of the *p*-chlorophenyl group and concomitant conversion of the 4-triazolyl moiety to uracil.[2] Then sequential treatments with ammonia, 80% acetic acid, and tetrabutylammonium fluoride as in the previous procedure yielded the third unprotected heptamer 5′-OH-AUUUA*UC*-2′,3′-OH.[10,19,20]

After purification by thin-layer chromatography on poly(ethyleneimine), the three deprotected heptamers were labeled with ^{32}P at the 5′-terminus by T_4 polynucleotide kinase. Their nucleotide sequences were confirmed by the two-dimensional mobility–shift analysis of the nuclease digest of the 5′-labeled heptamers (Figure 1).[21] Random spots that accompanied the major sequences may be caused by unexpected breakage of the base-sensitive internucleotidic phosphate bond during various elutions and the sequencing process.

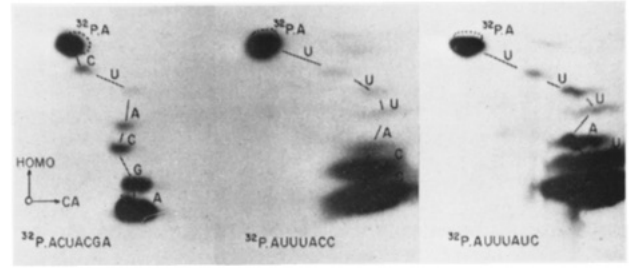
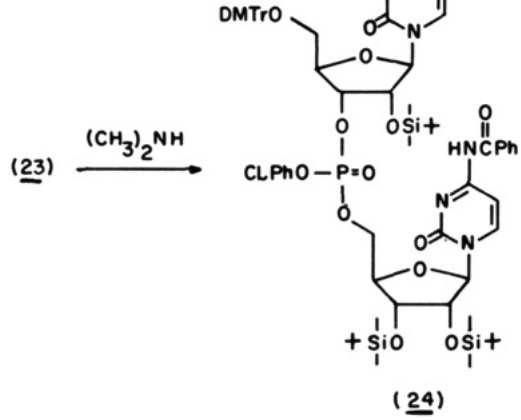


Figure 1. Two-dimensional chromatographic fingerprints of ^{32}P-labeled heptanucleotides after partial digestion with nuclease P_1 enzyme. The first dimension is electrophoresis on a cellulose acetate strip of pH 3.5 and the second dimension is homochromatography on a DEAE–cellulose plate in solvent Homo-V.

For studying the feasibility of synthesizing analogues from the 4-triazolylpyrimidinone-containing oligonucleotide, the fully protected dinucleotide **23** (UV maxima at 277, 310, and 321 nm) prepared earlier (Table I) was treated with dimethylamine briefly (eq 2). The 4-triazolyl



(2)

group was readily displaced by the amine to yield the fully protected N^4,N^4-dimethylcytosine analogue (**24**). Both

(19) M. D. Matteucci and M. H. Caruthers, *J. Am. Chem. Soc.*, **103**, 3185 (1981).
(20) K. K. Ogilvie, N. Y. Theriault, J.-M. Seifert, R. T. Pon, and M. J. Nemer, *Can. J. Chem.*, **58**, 2686 (1980).
(21) C. D. Tu and R. Wu, *Methods Enzymol.*, **65**, 620–630 (1980).

NMR (broad singlet of two N^4-methyl groups at δ 3.11) and UV (278 nm, ϵ 24 800) spectra of this dinucleotide suggested the structure designated.[11]

In conclusion, 4-triazolylpyrimidinone nucleotide *M* (**20**), as a "pyrimidine-to-pyrimidine" intermediate, has added immense flexibility in oligoribonucleotide synthesis.

Experimental Section

5′-*O*-(Dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)uridine (**21**) and the four common ribonucleotides (A, C, G, U) in the form of a 5′-*O*-(dimethoxytrityl)-*N*-acyl-2′-*O*-(*tert*-butyldimethylsilyl)-3′-*O*-[(*p*-chlorophenoxy)(cyanoethoxy)phosphoryl] triester were prepared via established procedure.[10,17] Tetramethylsilane was used as an internal reference for ^{1}H NMR. All reactions were conducted at room temperature unless otherwise stated.

1-(5′-*O*-(Dimethoxytrityl)-2′,3′-bis(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl)-4-(1,2,4-triazol-1-yl)-pyrimidin-2(1*H*)-one (12). *p*-Chlorophenyl phosphodichloridate (2.0 g, 8.1 mmol) was stirred in a solution of 5′-*O*-(dimethoxytrityl)-2′,3′-bis(*tert*-butyldimethylsilyl)uridine (**11**; 2.3 g, 3.0 mmol), 1,2,4-triazole (1.15 g, 16.6 mmol), and pyridine (10 mL) for 72 h and was extracted with water and dichloromethane. The organic extract was washed with 2% aqueous sodium bicarbonate, and the solvent was removed in vacuo. The residue was purified by short-column chromatography on silanized silica gel (40% water in acetone) to yield **12**: 1.6 g (65%); mp 108 °C; UV max (MeOH) 315 nm (ϵ 7400), 282 (4140), min 290 (3170); ^{1}H NMR ($CDCl_3$) δ 9.09 (1 H, s), 8.93 (1 H, d, J = 7 Hz), 7.92 (1 H, s), 6.38 (1 H, d, J = 7 Hz), 3.70 (6 H, s). Anal. Calcd for $C_{44}H_{59}N_5O_7Si_2$: C, 64.00; H, 7.15; N, 8.48. Found: C, 63.87; H, 7.17; N, 8.33.

5′-*O*-(Dimethoxytrityl)-2′,3′-*O*-bis(*tert*-butyldimethylsilyl)cytidine (13). (a) A solution of **12** (0.30 g, 0.36 mmol) and 20% aqueous ammonia (2 mL) in dioxane (6 mL) was stirred for 2 h. Removal of solvent in vacuo yielded a residue which was purified by chromatography on silica gel to give **13**: 0.26 g (94%); mp 154 °C; UV max (MeOH) 275 nm (ϵ 9420), min 259 (7500); ^{1}H NMR ($CDCl_3$) δ 8.36 (1 H, d, J = 7 Hz), 5.39 (1 H, d, J = 7 Hz), 3.88 (6 H, s). Anal. Calcd for $C_{42}H_{59}N_3O_7Si_2$: C, 65.20; H, 7.63; N, 5.43. Found: C, 65.01; H, 7.74; N, 5.61.

(b) 5′-*O*-(Dimethoxytrityl)-2′,3′-*O*-bis(*tert*-butyldimethylsilyl)-N^4-benzoylcytidine (200 mg, 0.23 mmol) was dissolved in a solution of 20% ammonium hydroxide (1 mL) and pyridine (4 mL) at 37 °C. After 72 h, solvent was evaporated in vacuo. Purification of the residue by chromatography on silica gel yielded **13** (152 mg, 86%) identical with a sample prepared in part a.

5′-*O*-(Dimethoxytrityl)-2′,3′-*O*-bis(*tert*-butyldimethylsilyl)-N^4-methylcytidine (14). A solution of **12** (200 mg, 0.24 mmol) and 40% aqueous methylamine (2 mL) in dioxane (4 mL) was stirred for 1 min. Removal of solvent in vacuo yielded a residue which was purified by chromatography on silica gel to give **14**: 171 mg (90%); mp 125 °C; UV max (MeOH) 277 nm (ϵ 12 600), min 260 (9800); ^{1}H NMR ($CDCl_3$) δ 8.15 (1 H, d, J = 7 Hz), 5.19 (1 H, d, J = 7 Hz), 3.69 (6 H, s), 2.78 (3 H, br s). Anal. Calcd for $C_{43}H_{61}N_3O_7Si_2$: C, 65.56; H, 7.75; N, 5.33. Found: C, 65.38; H, 7.80; N, 5.13.

5′-*O*-(Dimethoxytrityl)-2′,3′-*O*-bis(*tert*-butyldimethylsilyl)-N^4,N^4-dimethylcytidine (15). A solution of **12** (280 mg, 0.34 mmol) and 40% aqueous dimethylamine (2 mL) in dioxane (4 mL) was stirred for 1 min. Removal of solvent in vacuo yielded a residue which was purified by chromatography on silica gel to give **15**: 238 mg (88%); mp 97 °C; UV max (MeOH) 280 nm (ϵ 14 600), min 255 (8800); ^{1}H NMR ($CDCl_3$) δ 8.32 (1 H, d, J = 7 Hz), 5.21 (1 H, d, J = 7 Hz), 3.76 (6 H, s), 3.03 (6 H, br s). Anal. Calcd for $C_{44}H_{63}N_3O_7Si_2$: C, 65.92; H, 7.87; N, 5.24. Found: C, 65.51; H, 7.98; N, 5.41.

4-Hydrazino-1-[5′-*O*-(dimethoxytrityl)-2′,3′-*O*-bis(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]pyrimidin-2(1*H*)-one (16). A solution of **12** (250 mg, 0.30 mmol) and hydrazine (450 mg, 14 mmol) in dioxane (6 mL) was stirred for 30 min. Removal of solvent in vacuo yielded a residue which was purified by chromatography on silica gel to give **16**: 214 mg (90%); UV max (MeOH) 284 nm (ϵ 13 000), min 257 (8500); ^{1}H NMR ($CDCl_3$) δ 3.84 (6 H, s), 2.08 (3 H, s).

1-[2′,3′-*O*-Bis(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4-(1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (17). A solution of **12** (150 mg, 0.18 mmol) in dichloromethane (3 mL) was treated with 2% benzenesulfonic acid (4 mL, 30% methanol in dichloromethane) for 2 min and was neutralized by 2% pyridine in water. Evaporation of solvent from the organic extract yielded a residue which was purified by chromatography on silica gel to give **17**: 91 mg (96%); mp 88 °C; UV max (MeOH) 314 nm (ϵ 7440), min 279 (1830); ^{1}H NMR ($CDCl_3$) δ 9.27 (1 H, s), 8.79 (1 H, d, J = 7 Hz), 8.09 (1 H, s), 7.03 (1 H, d, J = 7 Hz). Anal. Calcd for $C_{23}H_{41}N_5O_5Si_2$: C, 52.77; H, 7.84; N, 13.38. Found: C, 52.53; H, 7.83; N, 12.99.

4-Methoxy-1-[2′,3′-*O*-bis(*tert*-butyldimethylsilyl-β-D-ribofuranosyl]pyrimidin-2(1*H*)-one (19). Compound **17** (410 mg, 0.84 mmol) was dissolved in a solution of 20% aqueous ammonia (3 mL) and methanol (10 mL). After 2 h, the solvent was evaporated. The residue was purified by chromatography on silica gel (2% acetone in dichloromethane) to give **18**[10] (25 mg, 6%) and the faster moving **19** (262 mg, 69%).

4-Methoxy-1-[2′,3′-*O*-bis(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]pyrimidin-2(1*H*)-one (**19**): mp 68 °C; UV max (MeOH) 276 nm (ϵ 6200), min 239 (1180); ^{1}H NMR ($CDCl_3$) δ 7.99 (1 H, d, J = 7 Hz), 5.90 (1 H, d, J = 7 Hz), 3.93 (3 H, s). Anal. Calcd for $C_{22}H_{42}N_2O_6Si_2$: C, 54.32; H, 8.64; N, 5.76. Found: C, 54.17; H, 8.71; N, 5.73.

Regeneration of 11 from 12 by Oximate Ion. Into a solution of **12** (200 mg, 0.24 mmol) in dioxane (4 mL) and water (0.2 mL) was added 0.5 M N^1,N^1,N^3,N^3-tetramethylguanidinium *syn*-2-pyridinealdoximate (2 mL, dioxane). After 2 h, the solvent was evaporated to give a residue which was purified by chromatography on silanized silica gel to give **11** (152 mg, 81%), identical with an authentic sample.

4-(1,2,4-Triazol-1-yl)-1-[5′-*O*-(dimethoxytrityl)-3′-*O*-((*p*-chlorophenoxy)(2-cyanoethoxy)phosphoryl)-2′-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]pyrimidin-2(1*H*)-one (*M*, 20). (a) 5′-*O*-(Dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)uridine (**21**; 6.1 g, 9.2 mmol), 1,2,4-triazole (3.22 g, 46 mmol), and *p*-chlorophenyl phosphodichloridate (5.6 g, 23 mmol) were stirred in pyridine (25 mL) for 48 h. β-Cyanoethanol (8 mL) was added. After 1 h, the mixture was diluted with water (100 mL) and extracted with dichloromethane (100 mL). The dichloromethane extract was washed with 2% aqueous bicarbonate (50 mL), and the solvent was removed in vacuo. The residue was purified by chromatography on silanized silica gel to give *M* (**20**): 6.3 g (72%); UV max (MeOH) 315 nm (ϵ 7500), 282 (4600), min 290 (3500); ^{1}H NMR ($CDCl_3$) δ 9.10 (1 H, s), 8.63 (1 H, d, J = 7 Hz), 7.95 (1 H, s), 6.30 (1 H, d, J = 7 Hz), 3.69 (6 H, s), 2.41 (2 H, m). Anal. Calcd $C_{47}H_{52}N_6O_{10}ClSiP$: C, 59.12; H, 5.45; N, 8.80. Found: C, 58.93; H, 5.59; N, 8.63.

(b) Triazole (62 mg, 0.88 mmol) and *p*-chlorophenyl phosphodichloridate (108 mg, 0.44 mmol) were added to a solution of **22** (200 mg, 0.22 mmol) in pyridine (1.5 mL). The solution was stirred for 48 h and was extracted with water and dichloromethane. The solvent was evaporated to give a residue which was purified by chromatography on silica gel to give *M* (**20**; 167 mg, 79%) identical with a sample prepared from part a.

General Method for Nucleotide Condensation. The procedure is similar to that reported for the synthesis of unmodified oligonucleotide.[17] As an example, the synthesis of [$(MeO)_2$Tr]-*M*±bzC-$(OSi)_2$ (see Table I for notation) is described. Modified nucleotide *M* (**20**; 400 mg, 0.42 mmol) was added to a solution of diisopropylamine (1 mL) and pyridine (4 mL). After 1 h, the solvent was evaporated to give the phosphorodiester.

5′-*O*-(Dimethoxytrityl)-2′,3′-*O*-bis(*tert*-butyldimethylsilyl)-N^4-benzoylcytidine (330 mg, 0.38 mmol) was treated with 2% benzenesulfonic acid (10 mL, 30% methanol in dichloromethane) for 2 min. The solution was neutralized with 2% pyridine in water. The solvent was removed from the organic extract to yield the detritylated cytidine derivative, which was then coevaporated with dichloromethane three times. This foamy residue was dissolved in pyridine (2 mL) and added into a mixture of mesitylenesulfonyl tetrazolide (140 mg, 0.51 mmol) and the phosphorodiester salt of *M* (**20**). After 15 min, the mixture was diluted with water and extracted with dichloromethane. The extract was washed with 2% aqueous sodium bicarbonate. Removal of solvent from the extract in vacuo yielded a residue which was purified by chromatography on silanized silica gel (40% water in acetone) to give the dinucleotide [$(MeO)_2$Tr]*M*±bzC-$(OSi)_2$ (**23**): 386 mg (69%);

UV max (MeOH) 321 nm (ε 5700), 310 (6770), 277 (20 600), min 266 (18 400); ^{1}H NMR ($CDCl_3$) δ 9.41 (1 H, s), 8.89 (1H, s), 3.87 (6 H, s).

Strategy and relevant data of the synthesis of the two heptamers AMUAMGA and AUUUAMC are presented in Table I.

Preparation of 5′-OH-ACUACGA-2′,3′-OH. Fully protected heptamer AMUAMGA (20 mg) was dissolved in a solution of 20% aqueous ammonia (1 mL) and dioxane (3 mL). After 8 h, the solvent was evaporated in vacuo. The residue was dissolved in 20% aqueous ammonia (2 mL) and pyridine (4 mL). After 4 days at 37 °C, the solvent was evaporated. The residue was washed with anhydrous ether and was treated at 20 °C for 15 min with acetic acid–water (4:1 v/v).[19] After evaporation of the solvent, the residue was washed with anhydrous ether and was treated with 1 M tetrabutylammonium fluoride in tetrahydrofuran (0.7 mL) for 3 h. After evaporation of solvent, the residue was dissolved in water (30 mL) and was filtered through a short column of [(diethylamino)ethyl]cellulose.[22] The column was then eluted by 1 M triethylammonium bicarbonate buffer (pH 7.2). Evaporation of the eluant yielded the unprotected heptamer ACUACGA. Further purification by TLC on poly(ethylenimine) afforded the heptamer (7 mg, approximately 70%) as an amorphous compound.[7]

Preparation of 5′-OH-AUUUACC-2′,3′-OH. Fully protected heptamer AUUUAMC (10 mg) was deprotected by the same procedure as in the preparation of ACUACGA. The resulting heptamer 5′-OH-AUUUACC-2′,3′-OH was isolated as an amorphous compound (4 mg, approximate yield 70%).

Preparation of 5′-OH-AUUUAUC-2′,3′-OH. Fully protected heptamer AUUUAMC (7 mg) was dissolved in a solution of N^1,N^1,N^3,N^3-tetramethylguanidinium *syn*-2-pyridinealdoximate (3.8 mg) in dioxane (1 mL) and water (0.1 mL). After 7 h, the solvent was evaporated to yield a residue which was dissolved in 20% aqueous ammonia (2 mL) and pyridine (4 mL). After 3 days at 37 °C, the solvent was evaporated, and the residue was washed with ethyl ether. Subsequent detritylation and then desilylation were similar to those described in the preparation of 5′-OH-ACUACGA-2′,3′-OH. Heptamer 5′-OH-AUUUAUC-2′,3′-OH was obtained as an amorphous compound (3 mg, approximate yield 70%).

***p*-Chlorophenyl Ester of 5′-*O*-(Dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-N^4,N^4-dimethylcytidylyl-(3′→5′)-2′,3′-*O*-bis(*tert*-butyldimethylsilyl)-N^4-benzoylcytidine (24).** A solution of dinucleotide **23** (150 mg, 0.10 mmol) and 40% aqueous dimethylamine (2 mL) in dioxane (4 mL) was stirred for 5 min. Removal of the solvent yielded a residue which was purified by chromatography on silica gel to give **24**: 115 mg (81%); UV max (MeOH) 278 nm (ε 24 800), 262 (18 000), min 255 (15 900); ^{1}H NMR ($CDCl_3$) δ 3.80 (6 H, s), 3.11 (6 H, br s).

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Registry No. **11**, 82444-76-6; **12**, 82456-20-0; **13**, 82444-77-7; **14**, 82444-78-8; **15**, 82444-79-9; **16**, 82456-21-1; **17**, 82444-80-2; **18**, 72409-47-3; **19**, 82444-81-3; *M* (**20**), 82444-82-4; *M* (**20**) phosphorodiester salt, 82444-85-7; **21**, 81246-80-2; **22**, 81265-94-3; **23**, 82444-83-5; **24**, 82444-84-6; 5′-OH-ACUACGA-2′,3′-OH, 82444-86-8; [$(MeO)_2$Tr]bzA±*M*±U±bzA±*M*±IsoG±bzA-$(OSi)_2$, 82494-74-4; 5′-OH-AUUUACC-2′,3′-OH, 82444-87-9; [$(MeO)_2$Tr]bzA±U±U±U±bzA±*M*±bzC-$(OSi)_2$, 82494-73-3; 5′-OH-AUUUAUC-2′,3′-OH, 82444-88-0; [$(MeO)_2$Tr]U±bzA-ClPh, 82468-98-2; [$(MeO)_2$Tr]bzA±U±U-ClPh, 82444-89-1; *M*±bzC-$(OSi)_2$, 82456-22-2; U±bzA±*M*±bzC-$(OSi)_2$, 82456-23-3; [$(MeO)_2$Tr]Uβ-bzA±*M*±bzC-$(OSi)_2$, 82456-24-4; [$(MeO)_2$Tr]bzA-ClPh, 82456-25-5; [$(MeO)_2$Tr]bzA±*M*-ClPh, 82444-90-4; [$(MeO)_2$Tr]bzA±*M*±U-ClPh, 82444-91-5; *M*±CE, 82444-92-6; U±CE, 82444-93-7; IsoG±bzA-$(OSi)_2$, 82444-94-8; bzA±*M*±IsoG±bzA-$(OSi)_2$, 82456-26-6; [$(MeO)_2$Tr]bzA±*M*±CE, 82444-95-9; [$(MeO)_2$Tr]bzA±*M*±U±CE, 82444-96-0; [$(MeO)_2$Tr]bzA±*M*±IsoG±bzA-$(OSi)_2$, 82468-99-3; *p*-Chlorophenyl phosphodichloridate, 772-79-2; 1,2,4-triazole, 288-88-0; 5′-*O*-(dimethoxytrityl)-2′,3′-*O*-bis(*tert*-butyldimethylsilyl)-N^4-benzoylcytidine, 81246-77-7; methylamine, 74-89-5; dimethylamine, 124-40-3; hydrazine, 302-01-2; 2′,3′-*O*-bis(*tert*-butyldimethylsilyl)-N^4-benzoylcytidine, 72409-40-6.

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Isolation and Structural Identification of 8(14),15-Sandaracopimaradiene-7α,18-diol from *Iboza riparia*

Norbert De Kimpe*[1] and Niceas Schamp

Laboratory of Organic Chemistry, Faculty of Agricultural Sciences, State University of Gent, Coupure Links 653, B-9000 Gent, Belgium

Luc Van Puyvelde,* Serge Dubé, and Monique Chagnon-Dubé

Centre Universitaire de Recherche sur la Pharmacopée et la Médicine Traditionelle, Curphametra, B.P. 52, Butare, Rwanda

Francois Borremans and Marc J. O. Anteunis

Laboratory of Organic Chemistry, NMR Spectroscopic Unit, Faculty of Sciences, State University of Gent, Krijgslaan 271 (S4), B-9000 Gent, Belgium

Jean-Pierre Declercq,[1] Gabriel Germain, and Maurice Van Meerssche

Laboratoire de Chimie Physique et de Cristallographie, Bâtiment Lavoisier, B-1348 Louvain-la-Neuve, Belgium

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The isolation from *Iboza riparia* and structural elucidation of 8(14),15-sandaracopimaradiene-7α,18-diol, a novel diterpene diol with interesting pharmaceutical properties, are described.

Iboza riparia (Hochst) N.E.Br. (Labiatae) is an important medicinal plant in Rwanda (Central Africa) and is commonly used by the native people.[2] Some constituents of the leaves have been described recently, including

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