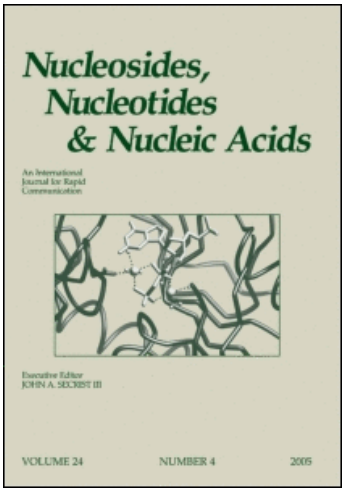

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Ozola, Vita , Ramzaeva, Natalya , Maurinsh, Yuris and Lidaks, Margeris(1993) 'Synthesis of 8-Substituted 7-(α-L-Arabinofuranosyl)theophyllines', Nucleosides, Nucleotides and Nucleic Acids, 12: 5, 479 — 486
To link to this Article: DOI: 10.1080/07328319308021217
URL: http://dx.doi.org/10.1080/07328319308021217

NUCLEOSIDES & NUCLEOTIDES, 12(5), 479-486 (1993)

SYNTHESIS OF 8-SUBSTITUTED 7-(α-L-ARABINOFURANOSYL)THEOPHYLLINES

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ABSTRACT: Glycosylation of trimethysilyl theophylline by 1,2,3,5-tetra-O-acetyl-L-arabinofuranose yielding 7-(α-L-arabinofuranosyl)theophylline after deacetylation is reported. A method for the synthesis of 8-chlorotheophylline α-L-arabinofuranoside using N-chlorosuccinimide was found. Further nucleophilic displacement of chlorine has provided the corresponding 8-amino- and 8-methylaminotheophylline α-L-arabinofuranosides.

Alkylxanthines and their 8-substituted derivatives, acting as adenosine antagonists, are known as compounds with broad spectrum biological activity: bronchospasmolytic, diuretic, cardiotonic, hypotensive, etc. [1]. Recently some reports have appeared on the ability of pentoxifylline (1-(5´-oxohexyl)-3,7-dimethylxanthine) to potentiate the antitumor effect of thiotepa[2] and to decrease the replication of the human immunodeficiency virus (HIV-1)[3]. Clinical trials of this compound in the first case are started[4] and are planned in the other [5].

Contrary to alkylxanthine bases, their nucleosides are less and irregularly studied both from the chemical and biological viewpoints with the main focus on the synthesis of glycosyl theophyllines [6-9]. Our recent paper concerning the synthesis of ß-D-glucofuranouronosides of theophylline and 3-

isobutyl-1-methylxanthine[10], as well as the latest report on the synthesis of ß-D-ribofuranosides of 1,3-dipropyl- and 1,3-dibutylxanthine[11], prompted us to obtain nucleosides modified both in purine and carbohydrate moiety: 8-substituted α-L-arabinofuranosides of theophylline.

Trimethylsilyl derivative of theophylline (**2**), obtained from theophylline (**1**) in the reaction with hexamethyldisilazane and $(NH_4)_2SO_4$, was glycosylated with 1,2,3,5-tetra-O-acetyl-α, ß-L- arabinofuranose (**3**) [12]. Compound (**3**) was used in preference to the recently reported 1,2-di-O-acetyl-3,5-di-O-benzoyl-α,ß-L-arabinofuranose [13,14] because of the more convenient preparation of **3** from L-arabinose in 85 % yield. The condensation reaction of **2** with **3** proceeded smoothly in 1,2 -dichloroethane (50°C, 1 h) with trimethylsilyl triflate as catalyst. The glycoside bond formation occurred according to the Tipson-Baker trans-rule [15] and only the desired 7-(2,3,5-tri-O-acetyl-α-L-arabinofuranosyl)theophylline (**4**) was obtained after flash chromatography on silica gel in 66 % yield. The other possible regio- and stereoisomers (7-ß- and 9-α/ß-arabinosides) were not found in the reaction mixture. Removal of the acetyl groups with methanolic ammonia afforded 7-(α-L-arabinofuranosyl)theophylline (**5**).

The UV spectrum of nucleoside **5** (λ_{max} 274 nm) was typical for N_7-glycosyltheophyllines [6-9]. In order to determine the anomeric configuration of nucleosides, NMR coupling constant of the H-1´ proton is commonly used. However, compound **5** shows a doublet for this proton with J = 4.2 Hz (Table), a value which is consistent with either an α- or a ß-configuration[16]. Therefore, this problem was solved by applying NOE difference spectroscopy, which has been proven as a reliable and convenient method for the assignment of the configuration at C-1 [17,18] in nucleosides. Saturation of the anomeric H-1´(6.02 ppm) caused an enhancement on the 5´-OH signal by NOE (4.86 ppm), whereas no change was observed for H-4´ (4.20 ppm). Thus, it is concluded that 5´-OH and H-1´ were on the same face of the sugar ring plane, e.g., compound **5** was assigned as the α-anomer.

Synthesis of 8-substituted purine nucleosides *via* their 8-halogeno derivatives (the most frequently 8-bromo) is the usual procedure. Unfortunately our attempts to introduce bromine into acetylated nucleoside **4** using N-bromoacetamide [19] or N-bromosuccinimide in chloroform or 1,2-dichloroethane failed even at the boiling temperature of the reaction mixture.

The routine procedure for C-8 bromination of unprotected purine nucleosides uses aqueous bromine at pH 4 [20] or bromine in acetate buffer at pH 6. Both methods, when applied to compound **5**, lead to rapid splitting of the glycosidic bond with release of theophylline (detected by TLC) for about 10 min.

In contrast to the bromination, chlorination of the acetylated theophylline nucleoside **4** with N-chlorosuccinimide in 1,2-dichloroethane proceeded smoothly at room temperature for 2 h, yielding, after flash chromatography on silica gel, 85 % of the desired 8-chloro derivative **6**. Deacetylation of **6** with methanolic ammonia or 25 % aqueous ammonia in methanol (1:1) afforded a mixture of the deacetylated nucleoside **7** and 8-aminotheophylline nucleoside **8** even at 0°C. Elevation of temperature increased the amount of compound **8** (TLC detected), and carrying out this reaction in methanolic ammonia (60°C, 10 h) allowed us to obtain compound **8** in 54 % yield. When using aqueous ammonia under the same conditions, the isolated yield of **8** was somewhat lower (48 %).

The UV spectrum of **8** shows λ_{max} 289 nm (H_2O), which is very close to that reported for 7-alkyl-8-aminotheophyllines [21] [λ_{max} 292 nm (EtOH)], but is considerably different from that reported for 7-alkyl-8-chlorotheophyllines [22] [λ_{max} 278 nm (EtOH)]. Structure **8** was also confirmed by ^{1}H-NMR data, where the resonance signal of the amino group was clearly detected at 7.02 ppm.

7-(α-L-Arabinofuranosyl)-8-chlorotheophylline (**7**) was prepared in 60 % yield after silica gel chromatography by treating **6** with triethylamine in water/ethanol solution (80°C, 5 h). In addition, compound **7** was obtained in 64 % yield after chromatographic purification by reacting deacetylated nucleoside **5** with N-chlorosuccinimide in 1,2-dichloroethane (40°C, 30 min,

then at room temperature 2 h). Physical data of compound **7** synthesized by both methods completely coincided. The UV spectrum of **7** [λ_{max} 279 nm (H_2O)] was identical with that of 7-alkyl-8-chlorotheophylline [22]. The structure of **7** was supported also by the ^{1}H-NMR spectral data and the mass spectral data [FAB, m/e 347 (M^+)].

Simultaneous deacetylation and displacement of chlorine with a methylamino group were accomplished after treating **6** with 25 % aqueous methylamine in ethanol (1:2, v/v). The yield of 7-(α-L-arabinofuranosyl)-8-methylamino theophylline **9** was 80 %.

Biological testing of the synthesized compounds is going on and the results will be reported elsewhere.

EXPERIMENTAL

UV Spectra were recorded on a Specord UV-VIS (Carl Zeiss). ^{1}H-NMR spectra were obtained on Bruker WH-90 and Bruker WM-360 instruments with tetramethylsilane as internal standard. The NOE measurements were made as described in reference 18 on a Bruker AC-250. Fast-atom bombardment (FAB) mass spectra were recorded on a KRATOS MS-50. Melting points were determined with a Boethius hot-stage micro-scope and are uncorrected. Thin layer chromatography (TLC) was carried out on silica gel Silufol UV-254 (Kavalier) by using the following solvent systems (v/v): A) chloroform-methanol, 4:1; B) chloroform-methanol, 9:1; C) chloroform-ethyl acetate, 1:1. Column chromatography was performed on silica gel L 100/160 (Chemapol).

7-(2,3,5-Tri-O-acetyl-α-L-arabinofuranosyl)theophylline (4).

A suspension of theophylline **1** (5.0 g; 27.7 mmol) and ammonium sulphate (20 mg) in hexamethyldisilazane (50 ml) was refluxed for 1 h. The reaction mixture was evaporated to dryness *in vacuo* and coevaporated twice with 20 ml xylene. The residue was dissolved in 1,2 -dichloroethane and treated with 1,2,3,5-tetra-O-acetyl-α,β-L-arabinofuranose **(3)** (8.82 g; 27.7 mmol) and trimethylsilyl triflate (5.96 ml; 33.3 mmol). The resulting solution was

heated at 50°C for 1 h, then poured with vigorous stirring into a $NaHCO_3$ (20 g) suspension in chloroform (300 ml). After being stirred for 1 h, a precipitate was filtered off and washed with chloroform (3 x 50 ml).The pooled filtrates were washed with saturated aq. $NaHCO_3$ (2 x 100 ml) and water (2 x 100 ml). After drying over anhydrous Na_2SO_4, the solvent was evaporated in vacuo. The resulting oil was purified by flash chromatography on silica gel with chloroform as eluent to yield crude **4**, which was purified on a silica gel column with benzene-diethyl ether (3:2) as eluent to give pure **4** (7.52 g; 62 %) as a foam; R_f 0.63 (system B); UV spectrum: λ_{max} (EtOH) 274 nm (ε 8200).

Anal. calc. for $C_{18}H_{22}N_4O_9$: C, 49.32; H, 5.06; N, 12.78. Found: C, 49.05; H, 5.04; N, 12.55.

7-(α-L-Arabinofuranosyl)theophylline (5).

Acetate **4** (10 g; 22.8 mmol) was dissolved in a saturated solution of ammonia in methanol (100 ml), and the reaction mixture was kept at room temperature for 12 h. The solvent was removed *in vacuo*, and the residue was crystallized from water to yield 5.7 g (80 %) of **5**; m.p. 160-162°C; R_f 0.34 (A); UV spectra: λ_{max} (0.1 N HCl) 274 nm (ε 9200), λ_{max} (H_2O) 274 nm (ε 9100); λ_{max} (0.1 N NaOH) 273 nm (ε 8800); FAB MS: m/e 313 (M^+).

Anal. calc. for $C_{12}H_{16}N_4O_6$: C, 46.15; H, 5.16; N, 17.94. Found: C, 46.32; H, 5.10; N, 17.66.

7-(2,3,5-Tri-O-α-L-arabinofuranosyl)-8-chlorotheophylline (6).

To a solution of compound **4** (1.65 g; 3.76 mmol) in 1,2-dichloroethane (10 ml) was added N-chlorosuccinimide (0.75 g; 7.5 mmol). After stirring the reaction at room temperature for 12 h, precipitated succinimide was filtered off. The filtrate was evaporated *in vacuo* and the residue was twice chromatographed on a silica gel column with chloroform-ethyl acetate (1:1) as eluent to obtain 1.5 g (85 %) of compound **6**; m.p. 156-157°C; R_f 0.27 (C); UV spectrum: λ max (EtOH) 281 nm (ε 9600).

Anal. calc. for $C_{18}H_{21}ClN4O_9$: C, 45.72; H, 4.48; N, 11.85. Found: C, 45.55; H, 4.47; N, 11.73.

7-(α-L-Arabinofuranosyl)-8-chlorotheophylline (7).

Method A: A solution of **6** (0.47 g; 1 mmol) and triethylamine (0.76 ml; 5.4 mmol) in 50 % aqueous ethanol (3 ml) was heated at 80°C for 5 h, cooled at room temperature , neutralized to pH 7 with acetic acid, and evaporated *in vacuo.* The residue was purified by flash chromatography on a silica gel column with chloroform-ethanol (4:1) as eluent. Trituration with ether and recrystallization from EtOH afforded the desired compound **7** (0.21 g; 60 %); m.p. 219-220°C; R_f 0.44 (A); UV spectra: λ_{max} (0.1HCl) 279 nm (ε 10900); λ_{max} (H_2O) 279 nm (ε 10700), λ_{max} (0.1 N NaOH) 281 nm (ε 10800). FAB m/e 347 (M^+).

Anal. calc. for $C_{12}H_{15}ClN_4O_6$: C, 41.56; H, 4.36; N, 16.11. Found: C, 41.63; H, 4.53; N, 15.75.

Method B: Compound **5** (0.2 g; 0.64 mmol) and N-chlorosuccinimide (0.1 g; 0.75 mmol) were suspended in acetonitrile (2 ml). The mixture was then stirred at 40°C for 30 min and at room temperature for 2 h. The precipitate was filtered off and crystallized from absolute ethanol to yield 0.14 g (64 %) of **7**, identical in all aspects to the substance obtained by Method A.

7-(α-L-Arabinofuranosyl)-8-aminotheophylline (8).

Compound **6** (0.4 g; 0.85 mmol) was dissolved in a saturated solution of ammonia in methanol and heated in a sealed tube at 60°C for 10 h. After cooling to room temperature, the solvent was evaporated and the residue absorbed on silica gel and dried *in vacuo.* The silica gel mass was put on the top of a silica gel column (50 cm^3), which was eluted with an ethanol gradient in chloroform (99:1 to 3:1; 200 ml of each). The fractions containing **8** were pooled, evaporated, and crystallized from ethanol to yield 0.15 g (54 %) of **8**; m.p.246-247°C. A further crystallization from ethanol gave the analytical sample of **8**; m.p. 247-248°C; R_f 0.21 (A); UV spectra: λ max (0.1 N HCl) 288 nm (ε 13600), λ max (H_2O) 289 nm (ε 14700), λ max (0.1 N NaOH) 290 nm (ε 17.600). FAB m/e 328 (M^+).

Anal. calc. for $C_{12}H_{17}N_5O_6$ x 0.5 H_2O: C, 42.86; H, 5.39; N, 20.82. Found: C, 42.81; H, 5.37; N, 20.48.

7-(α-L-Arabinofuranosyl)-8-methylaminotheophylline (9).

To a solution of compound **6** (1.0 g; 2.1 mmol) in ethanol (20 ml) was added 25 % methylamine water solution (10 ml). After 2 h at room temperature, the reaction mixture was evaporated *in vacuo*, and the residue was crystallized from ethanol/hexane to yield 0.58 g (80 %) of **9**; m.p. 234-235°C; R_f 0.36 (A); UV spectra: λ max (0.1 N HCl) 296 nm (ε 13700), λ max (H_2O) 298 nm (ε 17600), λ max (0.1 N NaOH) 299 nm (ε 12200). FAB m/e 342 (M^+).

Anal. calc. for $C_{13}H_{19}N_5O_6$ x H_2O: C, 43.45; H, 5.89; N, 19.49. Found: C, 43.68; H, 5.57; N, 19.22.

ACKNOWLEDGEMENTS

This work was supported by the grants No.441 and No. 442 from the Latvian Council of Science. Y.M. thanks to the Alexander von Humboldt-Stiftung (Bonn-Bad Godesberg, Germany) for financial support. The authors gratefully acknowledge Profs. F. Seela and H. Rosemeyer (Universität Osnabrück, Germany) for performing of NOE experiments, Prof. W. Pfleiderer (Universität Konstanz, Germany) for helpful advice in revising this manuscript, and Mrs. H. Bauer for preparation of this manuscript.

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Received 5/11/92
Accepted 3/26/93