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1,3-Dipolar cycloaddition of alkenes to 3′-azido-3′-deoxythymidine as a route to 3′-deoxythymidin-3′-yl derivatives

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1,3-Cycloaddition of acrylonitrile, acrylamide, vinyl acetate and allyl alcohol to azido group of 3′-azido-3′-deoxythymidine under mild conditions afforded the corresponding adducts which were tested as inhibitors of HIV reverse transcriptase.

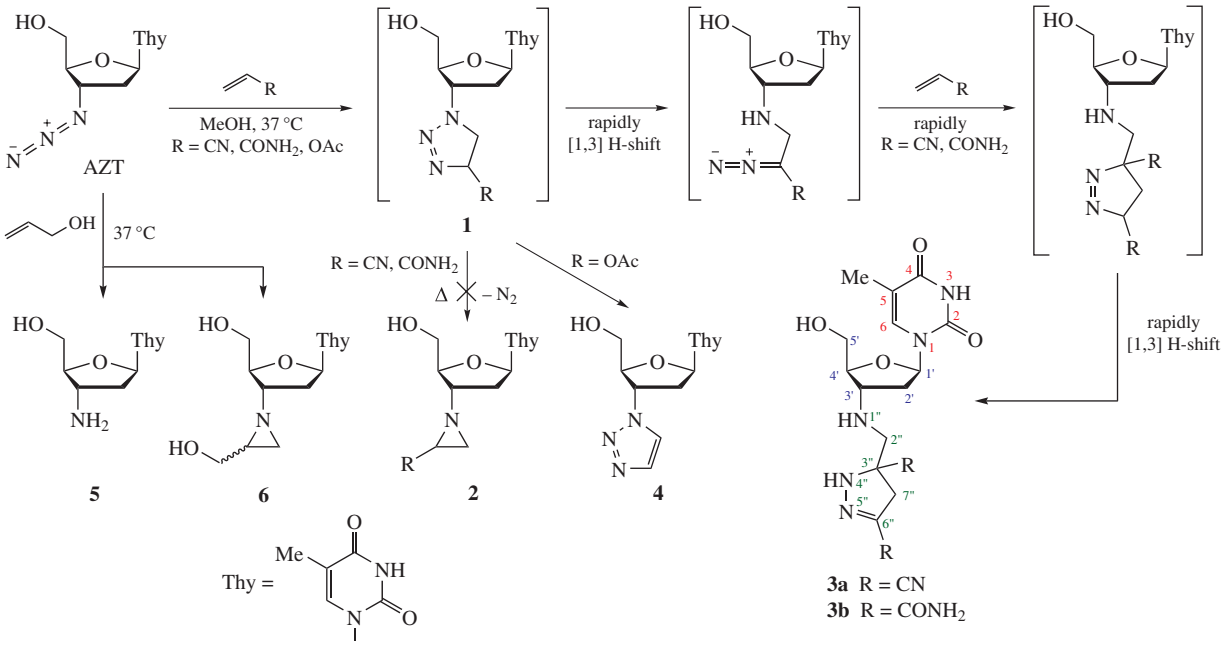
Thymidine is a nucleoside of a constant interest since the incorporation of different substituents in its 3′-position usually significantly changes its biological properties.[1] For instance, 3′-azido-3′-deoxythymidine (AZT) is the first and still the most applied nucleoside analogue in anti-HIV therapy. It has drawn our attention as a substrate for the Huisgen cycloaddition of different alkenes to azido group, resulting in derivatives with pyrazoline, aziridine and triazole substituents at 3′-position of 3′-deoxythymidine. 3′-Triazolyl-3′-deoxythymidine derivatives often become subjects of interest due to their biological activity. They were extensively studied in search for reverse transcriptase inhibitors[2] but had little activity against HIV; they were found to be effective inhibitors of human mitochondrial thymidine kinase;[3] several triazole derivatives prolonged life of mice carrying Erlich carcinoma. We concentrated our attention on obtaining under mild chemical conditions new thymidine derivatives which could have further application as anti-HIV drugs. Our methods require neither catalysis with transition metals (like 'click'-reactions of azides with alkynes[4]) nor stepwise staging with specific reagents. The target products have been obtained in moderate and good yields.

Azido group of AZT, being a dipole, is susceptible to cycloaddition with dipolarophiles,[5] among which acrylonitrile, acrylamide, vinyl acetate and allyl alcohol were selected for this study.

In pioneering articles by Gurvich[6] and Huisgen,[7] addition of azide to acrylonitrile gave Δ^2-1,2,3-triazolines, typical of further opening of N^2–N^3 bond, thus, resulting in a diazo intermediate. The nascent diazo compound is also a dipole capable of cycloaddition of one more acrylonitrile molecule (Scheme 1). Initially we intended to isolate the first step triazoline product by carrying out the reaction in a reasonable lack of the dipolarophile (0.7 equiv.) at 37 °C in methanol.† However, kinetically, the further ring opening of **1** and the second cycloaddition proceeded faster than the first stage of formation of **1** giving no possibility to detect **1** as a product. Moderate heating below 70 °C for 0.5–5 h did not favour the formation of aziridines **2**. As a result, the only product **3a** was isolated and studied, testifying that two molecules of acrylonitrile were added to azido group. Nitrile fragments were not involved in cycloaddition. This transformation was confirmed by UV and ^{1}H, ^{13}C NMR spectroscopy including combination of HH and CH correlations (COSY, HSQC, HMBC), as well as ^{15}N NMR and NH correlations. A product of the similar structure (**3b**) was obtained by cycloaddition of AZT and acrylamide though it required more time for the reaction.

† *3′-[(3,5-Dicyano-4,5-dihydro-1*H*-pyrazol-5-yl)methylamino]-3′-deoxythymidine* **3a**. Acrylonitrile (0.12 g, 2.25 mmol) was added to a solution of AZT (0.30 g, 1.12 mmol) in methanol (3 ml), stirred at room temperature, and the mixture was stirred for 20 h at 37 °C. The reaction mixture was concentrated, and the residue was chromatographed on a silica gel column (20×150 mm), eluted with a linear gradient of methanol (5% → 10%) in chloroform. The fraction containing the target product (a mixture of two diastereomers) was concentrated to afford 100 mg (25%) of **3a** as colourless viscous liquid, and was freeze-dried to white powder. ^{1}H NMR (DMSO-d_6) δ: 11.21 (s, 1H, N^3H), 9.39 (s, 1H, $N^{4''}$H), 7.75 (s, 1H, C^6H), 6.16 (dd, 1H, $C^{1'}$H, $^3J_{1',2'a}$ 6.2 Hz, $^3J_{1',2'b}$ 6.4 Hz), 5.04 (br. s, 1H, OH), 3.74 (m, 1H, $C^{4'}$H), 3.76–3.68 and 3.67–3.59 (m, 2H, $C^{5'}H^a$, $C^{5'}H^b$), 3.47 and 3.30 (2d, 2H, $C^{7''}H_2$, $^2J_{7''a,7''b}$ 17.3 Hz), 3.41 (m, 1H, $C^{3'}$H), 3.40 (br. s, 1H, $N^{1''}$H), 2.98 (m, 2H, $C^{2''}H_2$), 2.16 (m, 2H, $C^{2'}H_2$), 1.82 (s, 3H, Me). ^{13}C NMR (DMSO-d_6) δ: 163.78 (s, C^4), 150.40 (s, C^2), 136.21 (s, C^6), 121.68 and 121.65 (2s, $C^{6''}$), 119.70 (s, $C^{3''}$CN), 114.30 (s, $C^{6''}$CN), 109.15 (s, C^5), 85.04 (s, $C^{4'}$), 83.68 (s, $C^{1'}$), 64.19 (s, $C^{3''}$), 61.36 and 61.30 (2s, $C^{5'}$), 57.86 (s, $C^{3'}$), 51.83 (s, $C^{2''}$), 41.28 and 41.20 (2s, $C^{7''}$), 37.63 (s, $C^{2'}$), 12.23 (Me). ^{15}N NMR (DMSO-d_6) δ: –4.3 (s, $N^{5''}$), –113.4 (s, $C^{6''}$CN), –126.2 (s, $C^{3''}$CN), –222.5 (s, N^3H), –226.0 (s, $N^{4''}$H), –232.7 (s, N^1), –341.2 ($N^{1''}$H). UV (λ_{max}/nm): 269.9. MS, *m/z*: 125.1 (4.9%, Thy), 219.3 (14.7%), 345.3 (57.6%, M–N_2), 372.0 (11.7%, M–1), 408.1 (100.0%, M+^{35}Cl$^-$), 410.1 (32.0%, M+^{37}Cl$^-$), 780.9 (65.9%, 2M+^{35}Cl$^-$), 782.9 (21.2%, 2M+^{37}Cl$^-$).

*3′-{[3,5-Bis(aminocarbonyl)-4,5-dihydro-1*H*-pyrazol-5-yl]methylamino}-3′-deoxythymidine* **3b**. Acrylamide (0.2 mg, 2.8 mmol) was added to a solution of AZT (0.20 g, 0.75 mmol) in methanol (1 ml), stirred at room temperature, and the mixture was stirred for 20 h at 37 °C. The reaction mixture was concentrated, the residue was chromatographed on a silica gel column (20×150 mm), eluted with a linear gradient of methanol (5% → 20%) in chloroform. The fraction containing the target product (a mixture of two diastereomers) was concentrated to afford 120 mg (39%) of **3b** as colourless viscous liquid, and was freeze-dried to white powder. ^{1}H NMR (DMSO-d_6) δ: 11.19 (s, 1H, N^3H), 7.71 and 7.72 (q, 1H, C^6H, $^4J_{6,5\text{-Me}}$ 1.1 Hz), 7.69 and 7.66 (2s, 1H, $N^{4''}$H), 7.39 and 7.24 (2s, 2H, $C^{6''}CONH_2$), 7.21 and 7.01 (2s, 2H, $C^{3''}CONH_2$), 6.08 and 6.07 (2dd, 1H, $C^{1'}$H, $^3J_{1',2'a}$ 2.5 Hz, $^3J_{1',2'b}$ 6.2 Hz), 4.98 (m, 1H, OH), 3.66 (m, 1H, $C^{4'}$H), 3.58 (m, 2H, $C^{5'}$H), 3.26 (m, 1H, $C^{4'}$H), 2.88 (m, 2H, $C^{7''}$H), 2.69 and 2.65 (2d, 2H, $H^{2''}$, $^3J_{1'',2''}$ 19.7 Hz, $^3J_{1'',2''}$ 19.9 Hz), 2.07 (m, 2H, $H^{2'}$), 1.77 and 1.76 (2d, 3H, Me, $^4J_{5\text{-Me},6}$ 1.1 Hz). ^{13}C NMR (DMSO-d_6) δ: 174.99 (s, $C^{3''}$CO), 163.70 (s, $C^{6''}$CO), 163.58 (s, C^4), 150.34 (s, C^2), 144.55 and 144.46 (2s, $C^{6''}$), 136.20 and 136.17 (2s, C^6), 109.09 and 109.03 (2s, C^5), 85.22 and 85.11 (2s, $C^{4'}$), 83.70 (s, $C^{1'}$), 72.59 and 72.53 (2s, $C^{3''}$), 61.68 and 61.37 (2s, $C^{5'}$), 57.98 and 57.74 (2s, $C^{3'}$), 52.12 (s, $C^{2''}$), 39.39 (s, $C^{7''}$), 37.71 and 37.55 (2s, $C^{2'}$), 12.16 (s, Me). UV (λ_{max}/nm): 270.5. MS, *m/z*: 258.1 (9.0%), 284.1 (14.5%, M–Thy), 410.2 (24.5%, M+1), 432.2 (100.0%, M+Na^+), 444.2 (21.9%), 470.2 (7.3%), 517.3 (29.4%), 547.3 (9.8%), 568.4 (10.9%), 610.4 (126%).



Scheme 1

It is known that under microwave irradiation cycloadducts of vinyl acetate and azides release AcOH affording unsubstituted triazoles.[8] In our study vinyl acetate couples with AZT[‡] yielding 3′-(1,2,3-triazolo)thymidine **4** as the only product, no microwave assistance having been applied.

Addition with poorly reactive allyl alcohol proceeded in its excess as a solvent within 7 days.[§] Two major products were obtained: the reduction product 3′-amino-3′-deoxythymidine **5** and 3′-[(2-hydroxymethyl)aziridin-1-yl]-3′-deoxythymidine **6** (two diastereomers in equimolar ratio). The structure of compound **6** was confirmed by HRMS and a combination of 2D NMR techniques.

Two diastereomers are clearly observed in the ^{1}H NMR spectrum of **6**, which differentiate several signals of the furanose and nucleobase (Figure 1). They appeared due to a newly formed stereocenter at the second position of the aziridine ring. It is possible to observe diastereomers *via* TLC and isolate them separately by the silica gel column chromatography. Usually C-monosubstituted aziridines may be observed in NMR with the first order AMX or the second order ABX patterns for CH_2 group of the cycle.[9] At first sight, the situation observed for diastereomers of **6** resembles the AMX pattern. But enrichment with one diastereomer after chromatography dramatically simplifies the 3″-CH_2 pattern. The uncommon character of ^{1}H NMR spectrum of **6** is that here aziridine 3″-CH_2 fragment reveals itself as an AA′X degenerate system showing no J_{gem} in $CDCl_3$, while 4″-CH_2OH of the aziridinyl side chain clearly indicates a typical ABX system with geminal J 11.4 Hz and vicinal J 4.5 and 6.7 Hz. It is proper to mention that this effect may not occur if other solvents suitable for NMR are used.[10] The same effect in small ring heterocycles has only been reported for β-hydroxy episulfides with *erythro* configuration of the alcohol.[11]

The configuration of 3″-stereocenter was determined by the 3″-CH_a shift observed in the enriched form. Compared to the

‡ *3′-(1,2,3-Triazol-1-yl)-3′-deoxythymidine* **4**. Vinyl acetate (0.20 g, 2.3 mmol) was added to a solution of AZT (0.20 g, 0.75 mmol) in methanol (2 ml), stirred at room temperature, and the mixture was stirred for 7 h at 50 °C. The reaction mixture was concentrated, and the residue was chromatographed on a silica gel column (20×150 mm), eluted with a linear gradient of methanol (5% → 10%) in chloroform. The fraction containing the target product was concentrated to afford 170 mg (77%) of **4** as colourless viscous liquid, which was freeze-dried to white powder. ^{1}H NMR (DMSO-d_6) δ: 11.31 (s, 1H, N^{3}H), 8.30 (d, 1H, C$^{5''}$H$_{triazole}$, $^3J_{5'',4''}$ 1.1 Hz), 7.81 (q, 1H, C^{6}H, $^4J_{6,5\text{-Me}}$ 1.2 Hz), 7.79 (d, 1H, C$^{4''}$H$_{triazole}$, $^3J_{4'',5''}$ 1.1 Hz), 6.42 (dd, 1H, C$^{1'}$H, $^3J_{1',2'}$ 6.7 Hz), 5.40 (m, 1H, C$^{3'}$H), 5.24 (t, 1H, OH, $^3J_{5'\text{-OH},5'}$ 6.7 Hz), 4.22 (m, 1H, C$^{4'}$H), 3.67 (m, 2H, C$^{5'}$H), 2.70 (m, 2H, C$^{2'}$H), 1.81 (d, 3H, Me, $J_{5\text{-Me},6}$ 1.2 Hz). ^{13}C NMR (DMSO-d_6) δ: 163.66 (s, C^4), 150.38 (s, C^2), 136.18 (s, C^6), 133.47 (s, C$^{4''}$), 124.39 (s, C$^{5''}$), 109.55 (s, C^5), 84.47 (s, C$^{4'}$), 84.90 (s, C$^{1'}$), 60.68 (s, C$^{5'}$), 58.94 (s, C$^{3'}$), 37.16 (s, C$^{2'}$), 12.16 (s, Me). UV (λ_{max}/nm): 266.3. MS, *m/z*: 83.2 (11.4%), 279.0 (8.3%, M–N), 316.1 (21.7%, M+Na$^+$), 332.1 (9.8%), 374.2 (15.1%), 402.3 (28.8%), 426.2 (18.1%), 432.2 (45.6%), 433.3 (10.3%), 459.0 (19.4%), 467.2 (9.2%), 487.2 (15.9%), 490.3 (22.7%), 516.3 (9.4%), 599.3 (18.0%), 609.4 (100.0%, 2M+Na$^+$).

§ *3′-Amino-3′-deoxythymidine* **5** *and 3′-[(2-hydroxymethyl)aziridin-1-yl]-3′-deoxythymidine* **6**. A solution of AZT (0.30 g, 1.12 mmol) in allyl alcohol (5 ml) was stirred for 96 h at 37 °C. The reaction mixture was concentrated, and the residue was chromatographed on a silica gel column (20×150 mm), eluted with a linear gradient of methanol (5% → 50%) in chloroform. The fraction containing the target product **5** (eluted in 50% methanol in chloroform) was concentrated by evaporation *in vacuo* and applied on a LiChroprep RP-18 (40-63 μm, Merck). The additionally purified product was concentrated to afford 46 mg (19%) of **5** as colourless crystals, mp 160 °C in agreement with reported data.[15] ^{1}H NMR (DMSO-d_6) δ: 7.76 (s, 1H, C^{6}H), 6.08 (dd, 1H, C$^{1'}$H, $^3J_{1',2'a}$ 5.4 Hz, $^3J_{1',2'b}$ 6.4 Hz), 4.20–4.80 (br. s, 3H, NH_2 and OH), 3.65 (dd, 1H, C$^{5'}$H^a, $J_{5'a,4'}$ 3.0 Hz, $J_{5'a,5'b}$ 11.7 Hz), 3.57 (dd, 1H, C$^{5'}$H^b, $J_{5'b,4'}$ 3.6 Hz, $J_{5'a,5'b}$ 11.7 Hz), 3.53 (m, 1H, C$^{4'}$H), 3.43 (m, 2H, C$^{3'}$H), 2.03 (m, 2H, C$^{2'}$H), 1.77 (s, 3H, Me). ^{13}C NMR (DMSO-d_6) δ: 163.79 (s, C^4), 150.37 (s, C^2), 136.32 (s, C^6), 108.91 (s, C^5), 87.57 (s, C$^{4'}$), 83.45 (s, C$^{1'}$), 60.71 (s, C$^{5'}$), 50.72 (s, C$^{3'}$), 40.59 (s, C$^{2'}$), 12.18 (s, Me). UV (λ_{max}/nm): 266.8.

The fraction containing the target product **6** (eluted in 20% methanol in chloroform) was concentrated to afford 93 mg (28%) of **6** as colourless viscous liquid, and was freeze-dried to white powder. ^{1}H NMR (DMSO-d_6) δ: 7.71 and 7.70 (q, 1H, C^{6}H, $^4J_{6,5\text{-Me}}$ 1.2 Hz), 6.20 and 6.19 (2dd, 1H, C$^{1'}$H, $^3J_{1',2'a}$ 6.0 Hz, $^3J_{1',2'b}$ 7.2 Hz), 4.99 (br. s, 1H, C$^{5'}$OH), 4.65 (dd, 1H, C$^{4''}$OH, $^3J_{4''\text{-OH},4''a} = {}^3J_{4''\text{-OH},4''b}$ = 5.4 Hz), 3.95 and 3.86 (m, 1H, C$^{4'}$H), 3.57 (m, 2H, C$^{5'}$H), 3.38 (dd, 1H, C$^{4''}$H^a, $^3J_{4''a,2''}$ 4.6 Hz, $^2J_{4''a,4''b}$ 11.3 Hz), 3.18 (dd, 1H, C$^{4''}$H^b, $^3J_{4''a,2''}$ 6.7 Hz, $^2J_{4''b,4''a}$ 11.3 Hz), 2.22 (m, 1H, C$^{3'}$H), 2.07 (m, 2H, C$^{2'}$H), 1.77 (d, 3H, Me, $^4J_{6,5\text{-Me}}$ 1.2 Hz), 1.66 (m, 1H, C$^{2''}$H), 1.53 and 1.51 (2d, 1H, C$^{3''}$H^a, $^3J_{3''a,2''}$ 3.5 and 3.6 Hz, $^2J_{3''a,3''a'}$ 0 Hz), 1.38 (d, C$^{3''}$H$^{a'}$, $^3J_{3''a',2''}$ 6.3 Hz, $^2J_{3''a',3''a}$ 0 Hz). ^{13}C NMR (DMSO-d_6) δ: 163.74 (s, C^4), 150.49 and 150.46 (2s, C^2), 136.16 and 136.10 (2s, C^6), 109.27 and 109.20 (2s, C^5), 85.44 and 85.18 (2s, C$^{1'}$), 84.06 and 83.92 (2s, C$^{4'}$), 68.96 and 68.52 (2s, C$^{3'}$), 63.17 and 63.14 (2s, C$^{4''}$), 61.93 and 61.66 (2s, C$^{5'}$), 39.83 and 39.82 (2s, C$^{2''}$), 37.43 and 37.23 (2s, C$^{2'}$), 30.16 and 30.07 (2s, C$^{3''}$), 12.22 (s, Me). UV (λ_{max}/nm): 267.5. HRMS, *m/z*: 298.1397 (M+1), 320.1217 (M+Na$^+$), 595.2722 (2M+1), 617.2545 (2M+Na$^+$), 892.4047 (3M+1), 914.3870 (3M+Na$^+$).

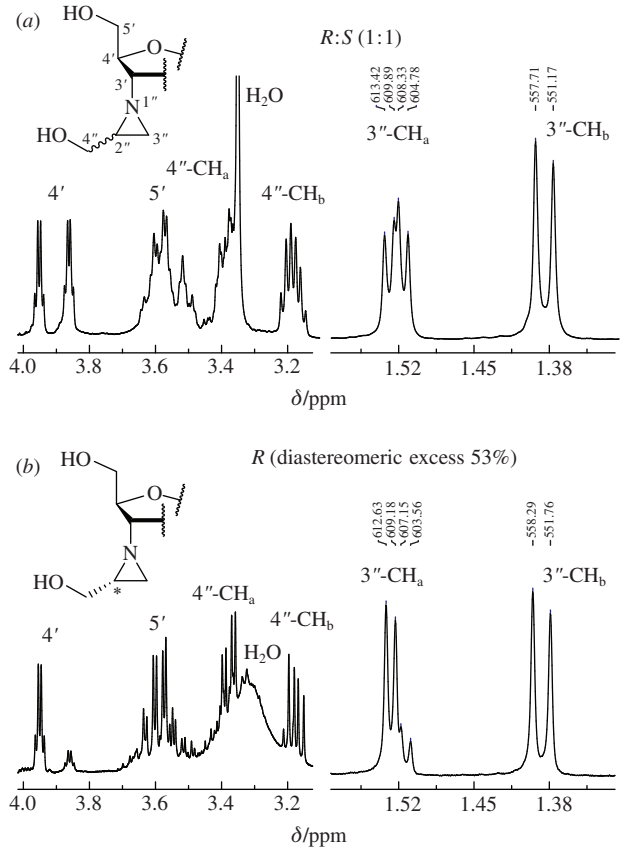


Figure 1 ^{1}H NMR spectra of compound **6**: (*a*) diastereomers in 1:1 ratio, (*b*) enriched with 3″-R isomer.

minor isomer this doublet is deshielded and the structure is known to be in *anti* configuration.[12] In addition, the conformation of the aziridine cycle in this diastereomer can be deduced: the 4′-CH downfield position shows that it is sterically compressed with the lone pair of aziridine nitrogen and therefore deshielded.[12] Aziridine is oriented away from the furanose cycle.

Although the overall yield of the products obtained by azide to alkene cycloadditions may be increased by the prolongation of the reaction time and raising the temperature,[7] we did not study the relationship between the yields and conditions in this work.

It was shown earlier that nucleoside derivatives can inhibit reverse transcriptase activity not only as chain terminators after their triphosphorylation (like AZT) but also as mimetics of non-nucleoside inhibitors, whose binding sites differ from that of substrate.[13] Bearing this in mind, we tested our 3′-thymidine derivatives as inhibitors in corresponding HIV-1 RT assays. However, they did not inhibit HIV RT at concentration up to 200 μM. Presumably, the inhibiting activity of pyrazoline derivatives could be expected in the corresponding triphosphorylated forms in a cell-free system.

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Online Supplementary Materials

Supplementary data associated with this article (^{1}H and ^{13}C NMR spectra for all the products, 2D NMR spectra for compounds **3a**, **4** and **6**; ^{15}N NMR spectra for **3a** and **6**, DFT energy calculations and orbitals visualization, HIV-1 reverse transcriptase assay procedure) can be found in the online version at doi:10.1016/j.mencom.2014.06.005.

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