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Biocatalytic Separation of *N*-7/*N*-9 Guanine Nucleosides

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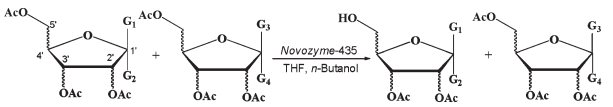

β-D-ribofuranosyl guanine:
G_1 = N^2-isobutanoylguanin-9-yl, G_2 = H, C-2' & C-3' OAc (α), C-4' CH_2OAc (β)
G_3 = N^2-isobutanoyl-7-yl, G_4 = H, C-2' & C-3' OAc (α), C-4' CH_2OAc (β)
α-D-arabinofuranosyl guanine:
G_1 = H, G_2 = N^2-isobutanoylguanin-9-yl, C-2' OAc (β), C-3' OAc (α), C-4' CH_2OAc (β)
G_3 = H, G_4 = N^2-isobutanoylguanin-7-yl, C-2' OAc (β), C-3' OAc (α), C-4' CH_2OAc (β)
α-L-arabinofuranosyl guanine:
G_1 = N^2-isobutanoylguanin-9-yl, G_2 = H, C-2' OAc (α), C-3' OAc (β), C-4' CH_2OAc (α)
G_3 = N^2-isobutanoylguanin-7-yl, G_4 = H, C-2' OAc (α), C-3' OAc (β), C-4' CH_2OAc (α)

Vorbrüggen coupling of trimethylsilylated 2-*N*-isobutanoylguanine with peracetylated pentofuranose derivatives generally gives inseparable *N*-7/*N*-9 glycosyl mixtures. We have shown that the *two* isomers can be separated biocatalytically by Novozyme-435-mediated selective deacetylation of the 5′-*O*-acetyl group of peracetylated *N*-9 guanine nucleosides.

The discovery of 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir) against herpes simplex type 1 and type 2 viruses and its low mammalian toxicity triggered the synthesis of a series of guanine nucleosides, e.g., penciclovir, famciclovir, valaciclovir, valganciclovir, abacavir, etc., for the treatment of various viral diseases.[1,2] The most problematic chemistry and difficulties in manipulation of all five common bases found in DNA and RNA occur with the polyfunctional guanine (pK_{a1} −1.7, pK_{a2} −9.2) nucleosides and nucleotides. The coupling of guanine with peracetylated sugar derivatives generally produces *N*-7/*N*-9 isomeric mixtures of nucleosides that are difficult to separate.[3,4] The changes in experimental variables and the use of a selectively modified guanine moiety, such as 2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine in nucleoside coupling reactions, affect the isomeric ratio but do not eliminate the formation of the *N*-7 isomer together with the desired *N*-9 isomer.[5] In this paper, we report the synthesis of guanine nucleosides (mixture of 9- and 7-glycosyl derivatives) derived from D-ribose, D-arabinose, and L-arabinose sugars and for the first time their highly efficient separation mediated by Novozyme-435 lipase-catalyzed removal of one of the acetoxy functions of the peracetylated *N*-9 guanine nucleosides.

The coupling of 2-*N*-isobutanoylguanine (**2**)[6] with 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose (**3**),[7,8] 1,2,3,5-tetra-*O*-acetyl-D-arabinofuranose (**4**),[9] or 1,2,3,5-tetra-*O*-acetyl-L-arabinofuranose (**5**)[8−10] in the presence of TMSOTf as Lewis acid catalyst following a standard Vorbrüggen[5,11] coupling protocol afforded mixtures of 2,3,5-tri-*O*-acetylated 9- and 7-β-D-ribofuranosylguanines **6** and **7**, 9- and 7-α-D-arabinofuranosylguanines **8** and **9**, and 9- and 7-α-L-arabinofuranosylguanines **10** and **11** in ratios of 87:13, 63:37, and 76:24, respectively, in 60−65% yields (Scheme 1 and Table 1). The ratio of regioisomers *N*-9 and *N*-7 in the above guanine nucleoside mixtures **6** and **7**, **8** and **9**, and **10** and **11** were calculated on the basis of the integration of the corresponding anomeric protons in the ^{1}H NMR spectra (400 MHz) of the mixtures (Table 1). Our various attempts of separation of *N*-9 and *N*-7 guanine nucleosides from the mixtures **6** and **7**, **8** and **9**, and **10** and **11** by repeated column chromatography on silica gel were unsuccessful.

Some lipases have been found to selectively acylate/deacylate primary hydroxyl over secondary hydroxyl group(s) of sugars[12]

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