DNA Primers used in this study (5’ to 3’)

14083
CACGGTCTCAATGAGTGATATCTTGAACGTAAGTCAACAACG
14084
GGAATTCCAATTATAATTCCCCCATTACGGCAGC
14082
GCTCTAGAGATGTGCTGTGACTGGGTTCC
14085
CGTGGAAGGATTTCAATGACTGTTAGTAAAATACCCATATGGCTAGATTG
14091
CGGGGTACCGAGACCATGGAGAACGGATACACCC
14092
GGAATTCCAAGGCAGGTCAACTGGC
14093
CGGGGTACCCTTTTAAGTTTATTCGGAGTTTCTCATGTGTTCTAG
14094
CCCAAGCTTCCCTTTTGAACTCCTTGGTAAAGTACG
14095
CGGGGTACCCATGGCCTCTGGCACCAC
14096
GGAATTCCAGTAGCTTTTGGCTTTATAGCGTTTGC

Plasmids used in this study

Name	Insert	Vector	Figures
pPAB003	*URH1*	pMalc2x	Tables 1
pPAB006	*PNP1*	pMalc2x	Tables 1
pPAB008	*Ppnp1-hMTAP*	pRS327	Figure 2
pPAB011	*Ppnp1-hPNP*	pRS327	Figure 2

***S. cerevisiae* strains used in this study**

Name	Genotype	Figures
BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0* (1)	5a, 6
PAB016	BY4742 *nrk1Δ::HIS3 pnp1Δ::kanMX4* (2)	6
PAB030	BY4742 *NRK1::TAP-HIS3MX6* (2)	4
PAB037	BY4742 *nrk1Δ::HIS3* (2)	2a
PAB038	BY4742 *pnp1Δ::kanMX4 urh1::NAT nrk1Δ::HIS3* (2)	6
PAB040	BY4742 *nrk1Δ::HIS3 urh1Δ::kanMX4* (2)	6
PAB052	BY4742 *pnp1Δ::kanMX4 urh1::NAT nrk1Δ::HIS3 meu1Δ::LEU2 ADH4::URA3-TEL* (2)	2b
PAB053	BY4742 *pnp1Δ::kanMX4 urh1::NAT nrk1Δ::HIS3 meu1Δ::LEU2* (2)	2a
PAB054	BY4742 *URH1:: TAP-HIS3MX6* (2)	4
PAB055	BY4742 *PNP1::TAP- HIS3MX6* (2)	4
PAB056	BY4742 *MEU1::TAP- HIS3MX6* (2)	4
JM84	BY4742 *ADH4::URA3-TEL* (2)	2b
JM85	BY4742 *nrk1⊿::HIS3*, *ADH4::URA3-TEL* (2)	2b
JM88	BY4742 *pnp1⊿::kanMX4 urh1⊿::NAT ADH4::URA3-TEL* (2)	2b
JM89	BY4742 *nrk1⊿::HIS3 pnp1⊿::kanMX4 urh1⊿::NAT ADH4::URA3-TEL* (2)	2b
KB038	BY4742 *bna1Δ::LEU2* (3)	5b

NaR/meNaR Syntheses

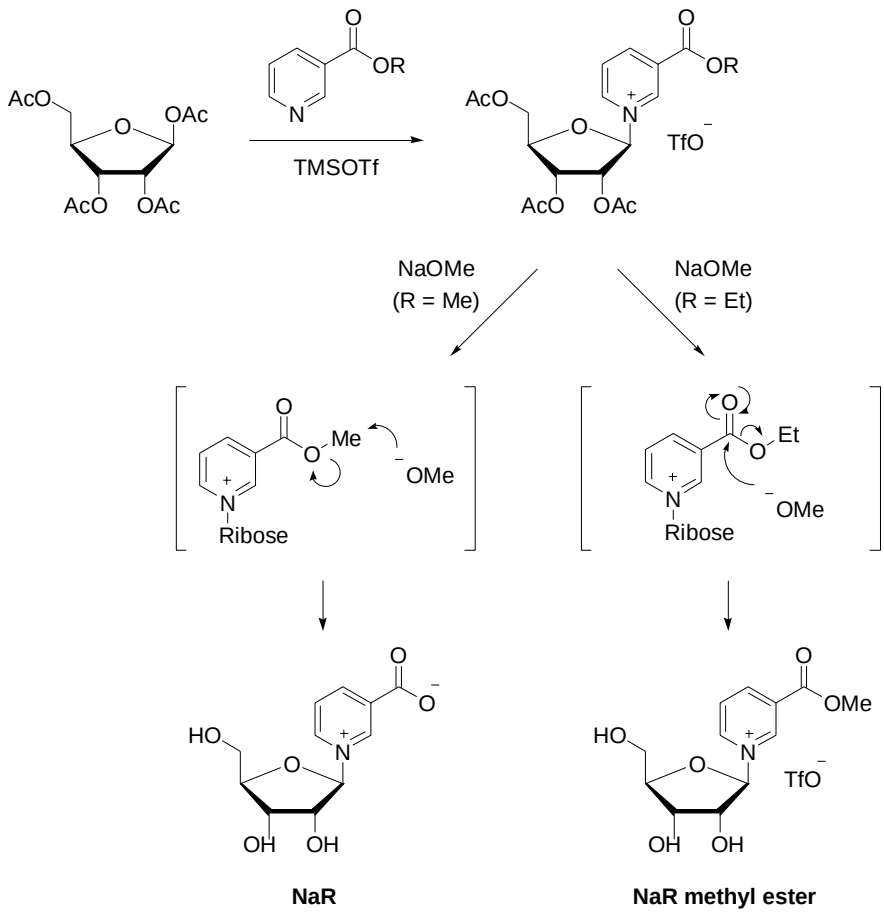
Syntheses of NaR and NaR ethyl ester have been described (4). We modified the procedure by using nicotinic acid methylester instead of nicotinic acid ethylester, to prepare both NaR and NaR methyl ester in the same synthetic sequence. Tetraacetyl ribose was reacted with excess of nicotinic acid methylester in presence of one equivalent of trimethylsilyl triflate to produce crude 2',3',5'-triacetyl NaR methylester triflate, which was deacetylated using three to four equivalents of sodium methoxide in methanol at -20 ^{o}C. At this point, the Yang et al. synthesis had produced crude meNaR, which was purified by HPLC. Our synthesis afforded a mixture of NaR and nicotinic acid methylester (the latter carried over from the first step) obviating the need for ester hydrolysis by pig liver esterase (4). The proton NMR spectrum of the crude product in D_2O exhibited only one methoxy group resonance at 3.94 ppm which increased when the sample was spiked with nicotinic acid methylester. HPLC purification of the crude product afforded pure NaR, the identity of which was confirmed by comparing its NMR spectrum with that of enzymatically prepared NaR.

We hypothesize that during the reaction, the methyl group of meNaR underwent nucleophilic attack by excess sodium methoxide (Supplementary Figure 1), which resulted in formation of the more thermodynamically stable zwitterionic NaR. Similar deprotection of esters by thiolates is well known and is commonly used as a mild method for converting methylesters into their corresponding carboxylic acids (5). The ethyl group in NaR ethyl ester is hindered enough to prevent this S_N2 reaction and that compound instead undergoes simple transesterification.

To produce stable meNaR, we reverted to using nicotinic acid ethylester in the reaction with tetraacetyl ribose. Deacetylation with sodium methoxide in methanol afforded crude meNaR. The 1H NMR spectrum of the crude product displayed two resonances due to methoxy group protons of meNaR (4.03 ppm) and nicotinic acid methylester (3.94 ppm). meNaR was then isolated from the crude mixture by HPLC.

Both synthetic NaR triflate and meNaR triflate were obtained stereospecifically as β-anomers as evidenced by the lone doublet of the anomeric proton (6.21 ppm for NaR and 6.25 ppm for meNaR).

Supplementary Figure 1



NaR **NaR methyl ester**

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