Tetrahedron Letters 55 (2014) 6204–6207

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Synthesis of non-hydrolyzable substrate analogs for Asp-tRNA^{Asn}/Glu-tRNA^{Gln} amidotransferase

Chayada Klinchan [a,b,†], Yu-Ling Hsu [b,†], Lee-Chiang Lo [b,*], Wanchai Pluempanupat [a], Pitak Chuawong [a,*]

[a] Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, and Special Research Unit for Advanced Magnetic Resonance (AMR), Kasetsart University, Bangkok 10900, Thailand
[b] Department of Chemistry, National Taiwan University, Taipei 106, Taiwan

ARTICLE INFO

Article history:
Received 4 July 2014
Revised 1 September 2014
Accepted 11 September 2014
Available online 18 September 2014

Keywords:
Adenosine
Substrate analog
Ketone hydrate
tRNA-dependent amidotransferase

ABSTRACT

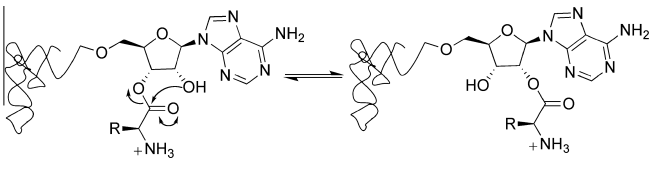
Non-hydrolyzable substrate analogs for tRNA-dependent amidotransferase, 2′- or 3′-aspartyl or -glutamyl adenosine, were synthesized from adenosine without protection of the adenine base. The hydroxyl groups of adenosine were selectively protected, followed by a series of oxidation/reductions to alter the stereochemistry. DFT calculations revealed the driving forces for the ketone hydrate formation at C-2′, but not the C-3′ carbon during the oxidation step. Subsequently, triflation and azide replacement yielded azidoadenosines, which were coupled to protected amino acids after deprotection and reduction. After global deprotection, the target substrate analogs were obtained in 2–14% overall yields from adenosine.

tRNA-dependent transamidation is a process in which the misaminoacylated tRNA(s), Glu-tRNA^{Gln} and/or Asp-tRNA^{Asn}, are converted into the correctly aminoacylated tRNA(s), Gln-tRNA^{Gln} and/or Asn-tRNA^{Asn}.[1] This process is shared by all archaea and some bacteria, especially pathogens such as *Staphylococcus aureus*[2] and *Helicobacter pylori*.[3] The heterotrimeric enzyme, Glu-tRNA^{Gln}/Asp-tRNA^{Asn} amidotransferase (GatCAB) is responsible for this biotransformation. Due to the absence of a GatCAB human homolog, this enzyme represents a potential target for antibacterial drug development. Recently, some transition state analogs as well as chloramphenicol derivatives were successfully tested in vitro as GatCAB inhibitors.[4,5]

Due to the spontaneous acyl shift at the ester bond connecting an amino acid to the cognate tRNA[6] (Scheme 1), the precise location of the amino acid during GatCAB catalysis is still unknown. Although it could be inferred from the crystal structure of the transamidosome[7] that the 3′-hydroxyl group on the adenosine is the amino acid attachment site, Glu-tRNA^{Gln}, generated from glutamyl-tRNA synthetase 2 (GluRS2) with the amino acid on the 2′-hydroxyl group, is also one of the substrates for GatCAB.[8] This intriguing substrate specificity in GatCAB prompted us to synthesize the four non-hydrolyzable substrate analogs shown in Figure 1.

These target molecules resemble the accepting base of Glu-tRNA^{Gln} and Asp-tRNA^{Asn} with Glu and Asp at the 2′ and 3′ positions of the adenosine, and connected through an amide bond rendering stability toward the acyl shift and hydrolysis. Investigation of the interactions between these small molecules and GatCAB should ultimately lead to a systematic design of small molecule inhibitors, which could be further developed into antibiotic drugs with novel mechanisms of action.

It is worth noting that the key step in the synthetic planning is to convert the 2′-/3′-hydroxyl group of adenosine into the corresponding nitrogen functionality with retention of the configuration. Initially, we envisaged the use of 2-acetoxyisobutyryl bromide to afford 2′- and 3′-bromoadenosine derivatives with β-configuration at the bromo substituent.[9,10] When the reaction was attempted, however, the bromoadenosine derivatives failed to react with various azide sources including sodium and TMS



Scheme 1. Spontaneous acyl shift in aminoacyl-tRNA.

* Corresponding authors. Tel.: +886 23 3661669; fax: +886 23 3668670 (L.-C.L.); tel.: +66 2 5625555x5161; fax: +66 2 5793955 (P.C.).
E-mail addresses: lclo@ntu.edu.tw (L.-C. Lo), Pitak.C@ku.ac.th (P. Chuawong).
† These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.tetlet.2014.09.060
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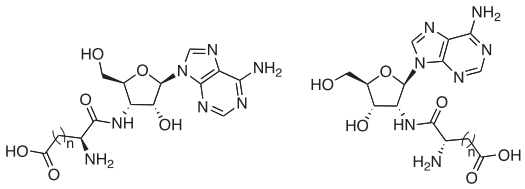


Figure 1. The non-hydrolyzable substrate analogs of GatCAB.

azide. In addition, previous reports in which bromoadenosines were successfully utilized as electrophiles are limited to nitrogen or oxygen nucleophiles, which reacted intramolecularly to afford cyclic intermediates.[11,12] We next shifted our attention to another double inversion strategy by utilizing an oxidation/reduction/substitution reaction sequence to first alter the stereochemistry of the 2′-/3′-hydroxyl groups and then to replace the activated hydroxyl groups with a nitrogen nucleophile (Scheme 2).

The synthesis started with partial protection of the adenosine hydroxyl groups using *tert*-butyldimethylsilyl chloride to give the 2′,5′- and 3′,5′-bis-*O*-*tert*-butyldimethylsilyladenosines in 45% (**2**) and 38% (**3**) yields, respectively.[13] The inversion of the configurations at the C2′ and C3′ carbons on the ribose moiety was carried out by an oxidation/reduction sequence. Initially, the oxidation was envisaged through the reaction between protected nucleosides and chromium trioxide (CrO_3) as previously reported.[14–17] In our case, however, the reaction proceeded with low product yield when the reported protocol was conducted, presumably due to the formation of a chromium-nucleoside complex, as suggested previously.[14] In addition, the chromatographic step was problematic, presumably because the chromium–nucleoside complex became trapped on the silica gel. It is worth mentioning that the same observation has been noted previously.[18] These complications impeded significantly the large-scale preparation of the keto-adenosine intermediates. With the problems related to chromic acid oxidation, along with its toxicity, alternative oxidants were investigated. The first alternative was explored using the Pfitzner–Moffatt (DMSO/DCC) oxidation protocol.[19] In this case, the desired product was generated in low yield. We then moved to the use of 2-iodoxybenzoic acid (IBX).[20,21] Unfortunately, the oxidation with this reagent did not proceed cleanly and the desired product was obtained in low yield. Finally, Dess–Martin periodinane (DMP) oxidized effectively both compounds **2** and **3** to afford compounds **4** and **11**, respectively, in good yields. The reactions were thus practical for scale-up, which certainly facilitated the subsequent modification steps.[15,22]

It is interesting to note that the 2′-ketoadenosine derivative **11** exists as an equilibrium mixture of the ketone and its hydrate form, which was spectroscopically confirmed using high-resolution mass spectrometry and NMR spectroscopy. The equilibrium was not observed with the 3′-ketoadenosine derivative **4**, which existed as a stable ketone. Our results agreed with a previous report in which the oxidation was conducted using the Pfitzner–Moffatt reagent.[19]

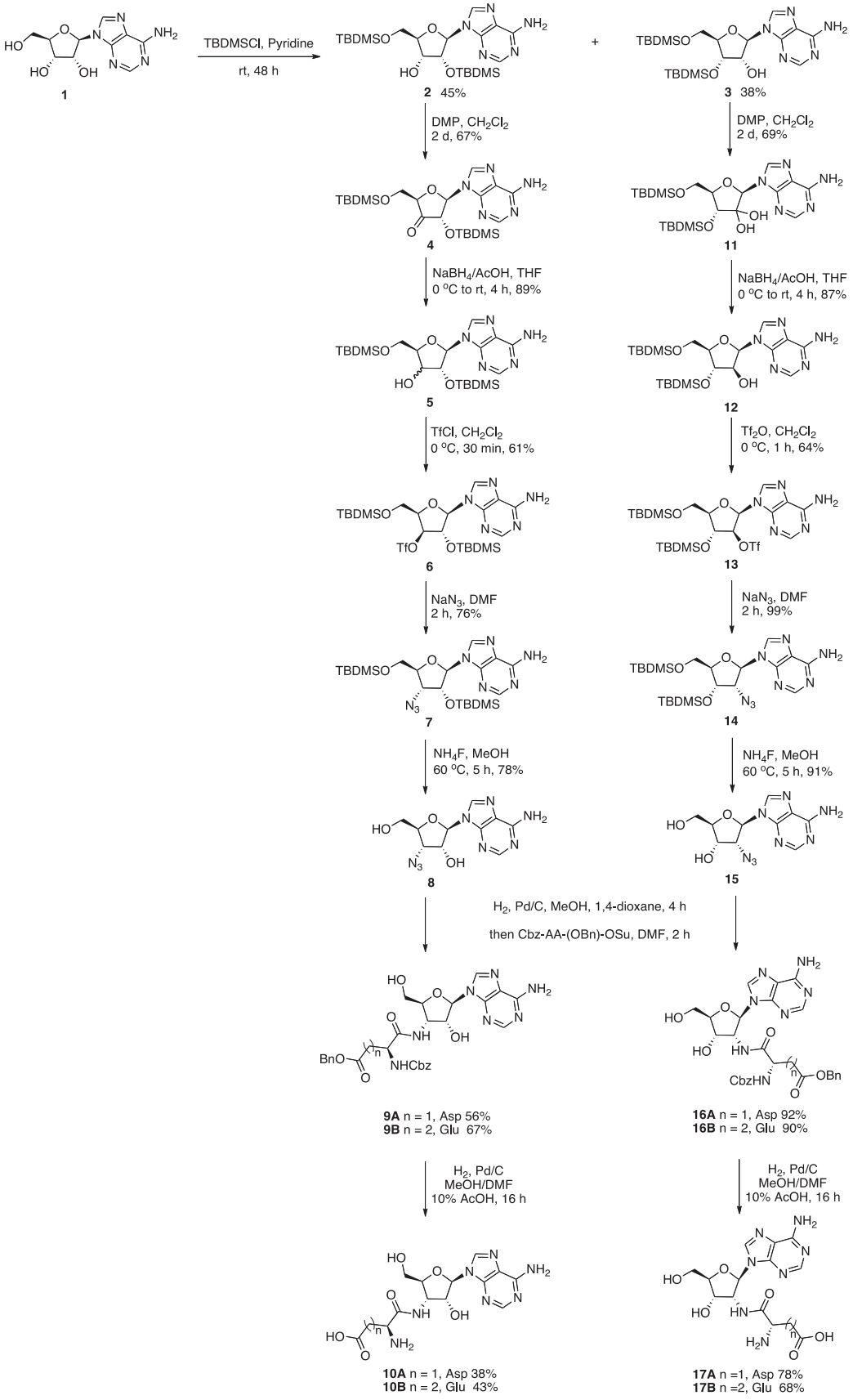
Due to these intriguing observations, we turned to computational chemistry for possible explanations. The DFT calculations using the B3LYP level of theory were set up for 2′- and 3′-ketoadenosine derivatives (both the ketone and hydrate form). The silyl protecting group was omitted in order to simplify the calculations. Although the total energy of the 2′- and 3′-ketone hydrates was not dramatically different, the geometries of these structures revealed an interesting piece of evidence. The N3 atom of the 2′-ketone hydrate was located very close to the hydroxyl group on the β-face at C2′ (the β-hydroxyl group) of the ribose ring. The distance of 2.00 Å between N3 and the hydrogen atom of the β-hydroxyl group stayed within the range of hydrogen bonding. This interaction is thought to pull the adenine ring toward the β-hydroxyl group in the optimized structure of the 2′-ketone hydrate (Fig. 2, panel A). On the other hand, the optimized structure of the 3′-ketone hydrate did not show any hydrogen bond interaction between the adenine base and the ribose (Fig. 2, panel B). Presumably, the observed internal hydrogen bonding between N3 and the β-hydroxyl group at the C2′ position in the 2′-ketone hydrate might be the driving force for the formation of the ketone hydrate at this position, while the 3′-isomer did not benefit from the same type of stabilization.

The reduction of compound **4** with excess sodium triacetoxyborohydride, generated in situ from $NaBH_4$ and AcOH at 0 °C, provided the *ribo* (minor) and *xylo* (major) diastereomers, as the hydride preferentially attacked the less sterically hindered α face. On the other hand, when similar conditions were applied to compound **11**, only the desired stereoisomer was obtained in high yield. In this case, the stereoselectivity was presumably induced by the steric hindrance of the adenine base located adjacent to the reaction center. After simple aqueous work-up, compounds **5** and **12** were used in the subsequent step without further purification. It is worth mentioning that the small amount of the undesired *ribo* isomer was eliminated during product purification in the next synthetic step.

For activation of the hydroxyl group at the 3′ position of the 2′,5′-bis-*O*-*tert*-butyldimethylsilyl adenosine, the previously reported conditions employing triflic anhydride (Tf_2O) resulted in a low product yield.[23] When the 2′-hydroxyl isomer **12** was subjected to Tf_2O in the presence of three equivalents of DMAP, we obtained the corresponding triflate **13** in good yield (64%). We also observed that the triflation reaction was sensitive to the solvent. No reaction was observed when THF was substituted for methylene chloride. Nevertheless, compound **5** failed to give the triflate product under these conditions. Instead, a smooth and rapid conversion of compound **5** into the triflate **6** was achieved using TfCl. In addition, we found that the reaction time could be shortened to less than one hour. Triflates **6** and **13** were then subjected to nucleophilic substitution with NaN_3 in DMF to afford azides **7** and **14** in good to excellent yields.

Deprotection of the silyl groups in compounds **7** and **14** was performed using NH_4F in MeOH at 60 °C.[24] The resulting alcohols **8** and **15** were obtained in good yields. Subsequently, the azido alcohols **8** and **15** were reduced using Pd/C and hydrogen gas. Without further purification, the resulting amines were directly coupled with the corresponding Cbz-protected L-amino acid derivatives to provide amide products **9A**, **9B**, **16A**, and **16B** in moderate yields. It is worth mentioning that the amino group on the adenine ring posed no threat to the coupling reaction. The final step involved hydrogenolysis to obtain the desired products (**10A**, **10B**, **17A**, and **17B**), which were conveniently purified using C-18 reverse phase chromatography.

In conclusion, non-hydrolyzable substrate analogs for Asp-$tRNA^{Asn}$/Glu-$tRNA^{Gln}$ amidotransferase (GatCAB) were synthesized with overall yields from 2% to 14%. The synthetic route reported herein does not require protection/deprotection of the amino group on the adenine moiety, and Dess–Martin periodinane was proven to be a suitable oxidant for the conversion of both the 2′- and 3′-hydroxyl groups of the ribose into the keto-adenosine intermediates, and applicable for large scale synthesis. The DFT calculations suggest the origin of the formation of the 2′-ketone hydrate via the internal hydrogen bond network, which was not observed in the 3′-keto isomer. Investigations on the interactions between the GatCAB enzyme and these non-hydrolyzable substrate analogs are in progress.



Scheme 2. Synthesis of the non-hydrolyzable substrate analogs for GatCAB.

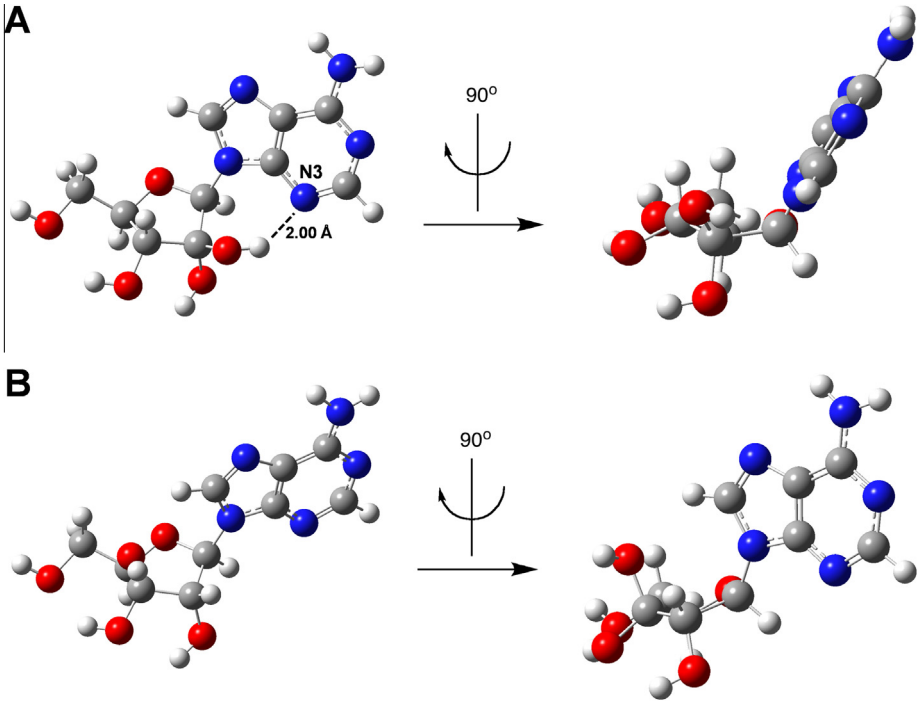


Figure 2. Optimized hydrate structure of **(A)** 2′-ketoadenosine and **(B)** 3′-ketoadenosine from the DFT calculations using the B3LYP level of theory.

Acknowledgments

We thank the Kasetsart University Research and Development Institute (KURDI) and the Faculty of Science, Kasetsart University. C.K. thanks the Office of the Higher Education Commission, Ministry of Education, and Kamphaen Phet Rajabhat University. LCL thanks the National Science Council of Taiwan (NSC 101-2113-M-002-007, NSC 102-2113-M-002-004-MY3). Financial support from the Center of Excellence for Innovation in Chemistry (PERCH-CIC), the Office of the Higher Education Commission, Ministry of Education is also gratefully acknowledged.

Supplementary data

Supplementary data (energy and coordinates for calculated structures, experimental details, copies of ^{1}H and ^{13}C spectra, and characterization data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.09.060.

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