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Facile rearrangement of N^4-(α-aminoacyl)cytidines to *N*-(4-cytidinyl)amino acid amides

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Abstract

Under near neutral and mildly basic conditions, primary N^4-(α-aminoacyl)cytidines (**4a**–**g**) undergo a facile rearrangement to form *N*-(4-cytidinyl)amino acid amides (**5a**–**g**). Secondary aminoacyl derivatives rearrange with other competing pathways. Tertiary aminoacyl derivatives do not rearrange.

Keywords: Rearrangement; N^4-(α-Aminoacyl)cytidines; *N*-(4-Cytidinyl)amino acid amides; Nucleoside

Nucleosides and their analogues are an important class of anticancer and antiviral agents. However, poor biopharmaceutical properties and an unfavorable therapeutic index often limit their use as therapeutic agents.[1] Recently, prodrug strategies have been utilized to improve the pharmacological properties of nucleoside analogues and to reduce toxic side effects of the parent molecules.[2] Amidon and co-workers have prepared amino acid ester prodrugs of various nucleoside analogues.[3] Wipf reported the synthesis of N^4-peptidyl-*ara*-C derivatives as chemoreversible prodrugs of *ara*-C.[4] In the 1960 s, Chheda's group reported that N^6-glycyladenine rapidly underwent a Dimroth rearrangement to *N*-(6-purinyl)glycine in aqueous solution at near neutral pH.[5] They further demonstrated that 9-alkyl-N^6-(aminoacyl)adenine derivatives can undergo a similar reaction to produce *N*-[6-(9-alkylpurinyl)]glycine.[6] In contrast, Stella and co-workers[7] reported in 1994 that aminoacyl amide prodrugs of prazosin undergo an intramolecular rearrangement involving the α-amine of the amino acid forming a spiro intermediate that opens to the α-alkyl amides. No citations of this report have appeared in the literature. However, two publications[8] and three patents[9] on aminoacyl amides of cytosine nucleosides isolated as HCl or TFA salts have appeared.

Amidon[3d] recently published the synthesis of water soluble amino acid ester prodrugs of gemcitabine (Gemzar®) (**1**), an effective anticancer drug widely used for pancreatic and non-small cell lung cancers.[10] Due to our interest in potentially more stable water soluble prodrugs of gemcitabine, a number of aminoacyl amides of gemcitabine were selected to synthesize. The results reported by Stella and co-workers[7] were of interest and in contrast to the reports on the aminoacyl amides of cytosine.[8,9] Therefore, we felt it important to utilize X-ray data to confirm structures. The synthesis of aminoacyl-gemcitabines **4a**–**g** is outlined in Scheme 1. Thus, the amino acid amides **3a**–**g** were prepared through a PyBOP/HOAt mediated selective acylation at the N^4-position of **1** using Boc-protected amino acids. Removal of the Boc-protecting group with TFA/CH_2Cl_2 (1:1) afforded the TFA salts of the corresponding N^4-(α-aminoacyl)gemcitabines (**4a**–**g**). The L-proline derivative **4h** was prepared in a similar fashion. To synthesize N^4-(*N*,*N*-dimethylphenylalanyl)gemcitabine **8** (Scheme 2), the

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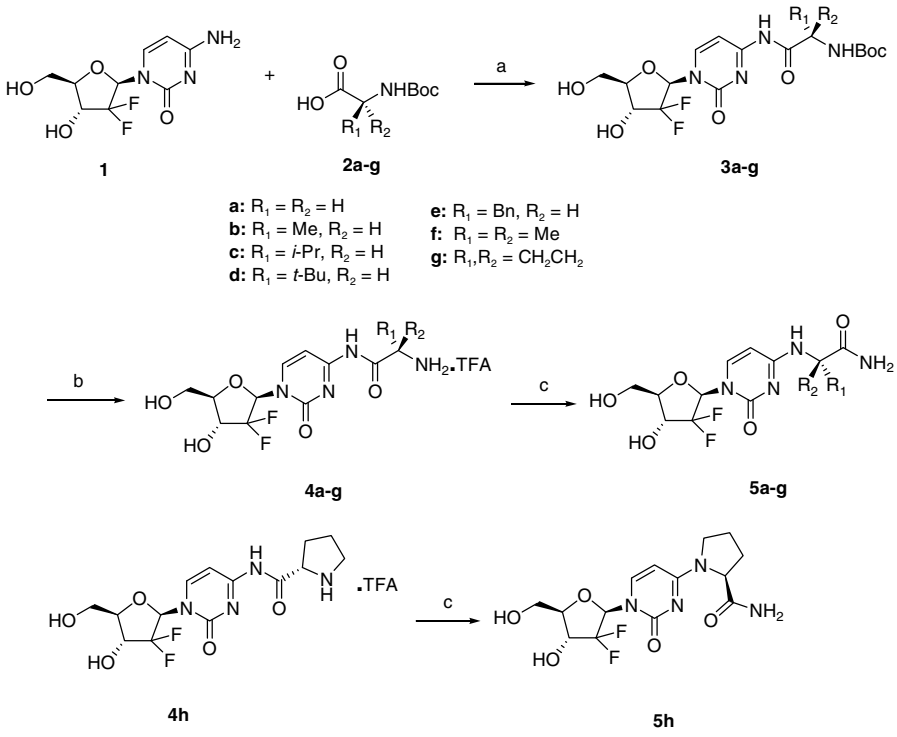
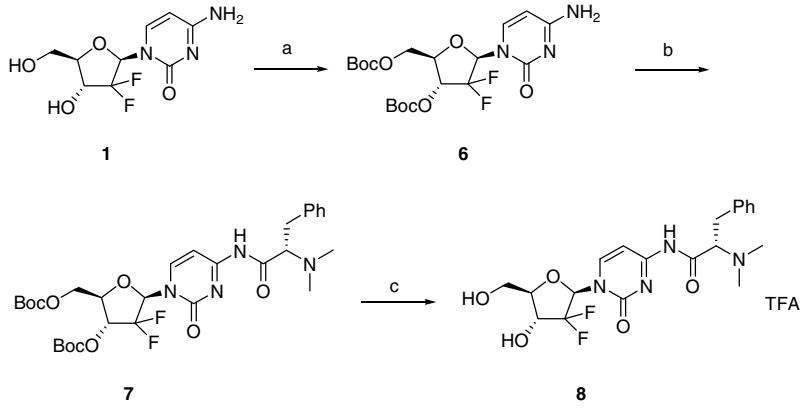


Scheme 1. Reagents: (a) PyBOP, HOAt, Et_3N, DMF; (b) TFA, CH_2Cl_2 (1:1); (c) saturated aqueous $NaHCO_3$, MeOH (1:1, v/v).



Scheme 2. Reagents: (a) (i) dioxane, 1 M aqueous KOH (1:1, v/v), Boc_2O (5 equiv); (ii) dioxane, Boc_2O (5 equiv), then 1 M aqueous KOH (1:1 to dioxane); (b) PyBOP, HOAt, Et_3N, DMF; (c) TFA, CH_2Cl_2 (1:1, v/v).

3′ and 5′-hydroxy groups of gemcitabine were selectively protected to obtain compound **6**. Coupling of **6** with *N*,*N*-dimethylphenylalanine under PyBOP/HOAt conditions afforded amide **7**. Removal of the protecting group provided **8** as the TFA salt.

Interestingly, compound **4d** with the bulky *t*-butyl group was 100% degraded at pH 8 at 40 °C in 4 h to a water insoluble product. While some amide hydrolysis to gemcitabine occurred under acidic conditions (pH 1–2), the observed degradation product at pH 8 was inconsistent with simple hydrolysis to gemicitabine. Comparison of the UV spectra of this product and **4d** indicated a change in electron density in the cytosine moiety (Fig. 1). Treatment of **4d** with a mixture of saturated aqueous $NaHCO_3$ and MeOH (Scheme 1) provided a single new compound (**5d**) with the same molecular weight as **4d** that exhibited a bathochromic shift in the UV spectrum (see Fig. 1).

The structure of **5d** was assigned based on both 1-D ^1H and 2-D homonuclear ^1H and heteronuclear ^1H–^{13}C NMR experiments.[11] The significant long-range correlations observed in the HMBC experiment are also shown in Figure. 2. A single crystal X-ray on the HCl salt of **5d** confirmed the structure (Fig. 3).[12] Similar studies using aqueous $NaHCO_3$–MeOH were performed on additional N^4-(aminoacyl)gemcitabines and the results are summarized in Table 1. Compounds **4a**–**h** were found to undergo rearrangement to **5a**–**5h**. The amide bond on the glycyl analog **4a** was very labile. Products arising from both

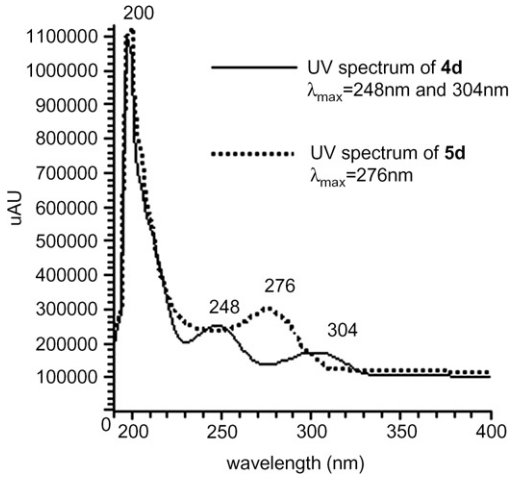


Fig. 1. Overlay of UV spectra of **4d** and **5d**.

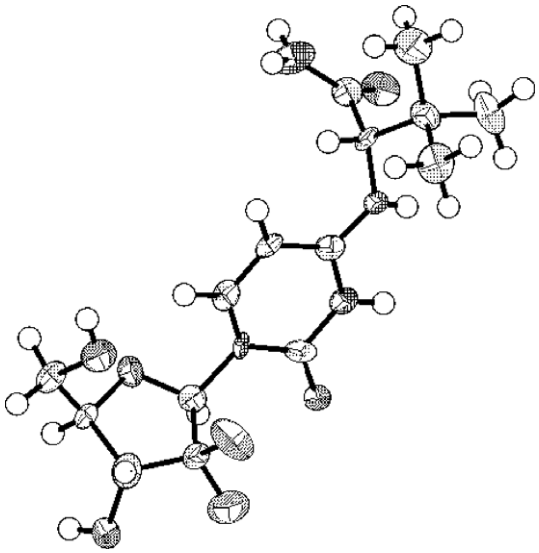


Fig. 2. ORTEP drawing of **5d**.

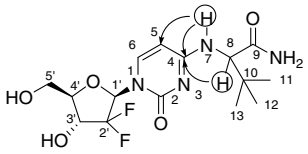


Fig. 3. Structure of **5d** with HMBC correlations.

Table 1
Results from rearrangement reactions

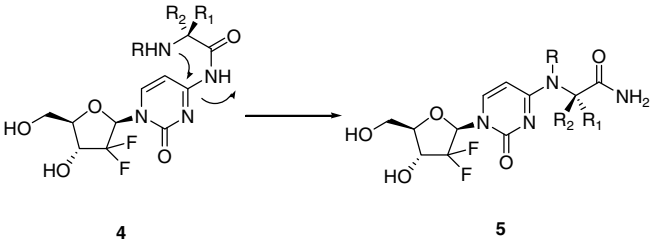
Product	Amino acid moiety	Rearrangement yield (%)
5a	L-Glycyl	51
5b	L-Alanyl	75
5c	L-Valyl	64
5d	L-*t*-Leucyl	78
5e	L-Phenylalanyl	81
5f	α,α-Dimethylglycyl	52
5g	α-Aminocyclopropylcarboxyl	69
5h	L-Prolyl	38
8	*N*,*N*-Dimethyl-L-phenylalanyl	N/A

hydrolysis to gemcitabine and rearrangement to **5a** were observed. Increasing the steric bulk at the α-position (**4b**–**g**) improved the hydrolytic stability of the aminoacyl derivatives and afforded the rearrangement product in good yields (64–81%) with the exception of α,α-dimethyl glycyl amide (**4f**) that provided **5f** in 52% yield.

It is interesting to note that the proline derivative **4h** underwent rearrangement similar to the open-chain analogs providing **5h**, albeit in 38% yield. The *N*,*N*-dimethyl derivative **8**, when subjected to the same reaction conditions was found to not undergo rearrangement but instead degrades to several products including gemcitabine. These results indicate the involvement of the amino group in the N^4-(α-aminoacyl)-gemcitabine degradation process. Compounds **4a**–**4g** that contain a free primary α-amino group primarily followed the rearrangement pathway. Under the reaction conditions, variation in the pK_a values of the amino acids was not a major factor as demonstrated by the conversion of **4g**–**5g**.

However, steric effects do contribute to the change in degradation pathways. Increasing the steric bulk of the α-substituent facilitated the rearrangement while increasing the bulkiness at the amino group (**4h**) slowed the rearrangement process and instead, favored the hydrolytic pathway. A mechanism for this rearrangement similar to the one proposed by Stella and co-workers[7] for the aminoacyl prazosin analogs is in Scheme 3. The reaction involves a nucleophilc attack of the free α-amino group in **4** at the 4-position of the cytidine ring, resulting in the displacement of the amide nitrogen.

In summary, N^4-(α-aminoacyl)gemcitabines are not stable in solution and undergo rapid pH dependent degradation. Under near neutral and basic conditions, primary N^4-(α-aminoacyl)-gemcitabines rearrange to novel *N*-(4-gemcitabinyl)amino acid amides that are no longer of interest as soluble prodrugs of gemcitabine. For secondary gemcitabine derivatives rearrangement is accompanied by amide hydrolysis. At low pH or when a tertiary amino acid moiety is present, the hydrolytic pathway predominates. This novel rearrangement process is germane to reports[8,9] of aminoacylamide cytosine nucleosides and would limit their in vivo utility. Equally important is the potential applicability of this rearrangement to other aminoacyl nucleosides.



Scheme 3. Proposed mechanism for the rearrangement reaction.

Acknowledgements

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11. *Compound* **5d**: ^{1}H NMR (600, DMSO-d_6) δ 0.95 (s, 9, *t*-Bu), 3.61 (m, 1, H5′a), 3.76 (m, 1, H5′b), 3.78 (m, 1, H4′), 4.14 (m, 1, H3′), 4.60 (d, 1, $J = 9.6$ Hz, H8), 5.19 (t, 1, $J = 5.5$ Hz, 5′-OH), 6.12 (t, 1, $J = 9.6$ Hz, H1′), 6.16 (d, 1, $J = 7.6$ Hz, H5), 6.22 (d, 1, $J = 6.6$ Hz, 3′-OH), 7.08 (s, 2, 9-NH_2), 7.62 (s, 1, 9-NH_2), 7.65 (d, 1, $J = 7.6$ Hz, H6), 7.85 (d, 1, $J = 9.6$ Hz, H7).
12. Crystallographic data (excluding structure factors) for **5d** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 622780. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).