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Novel Prodrug Approach to Amprenavir-Based HIV-1 Protease Inhibitors Via O→*N Acyloxy* Migration of P1 Moiety

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Abstract—We have developed a new approach to prodrugs, which utilizes a pH-induced intramolecular O→*N* migration of an acyloxy group in carbonate moiety to a free amino moiety at neutral pH. This method is exemplified by facile rearrangement of highly water-soluble prodrug **3** to carbamate **4**, a close analogue of HIV-1 protease inhibitor Amprenavir. The O→*N acyloxy* migration is unprecedented in the context of prodrugs and it enables a high atom economy due to recycling of the 'pro' moiety.

HIV-1 protease inhibitors (PI), such as Amprenavir **1**,[1] are key components in the highly active antiretroviral therapy (HAART), which has been credited with decreasing mortality rates from AIDS in the developed world. However, a heavy pill burden of HAART sometimes results in a limited patient adherence to the drug regimen, thus reducing or limiting the benefits of HAART and allowing emergence of viral resistance, rebound of viral load, and ultimately a disease progression. Much of the pill burden associated with PIs is the result of their generally low aqueous solubility. Consequently, there is a continued need to discover PIs with superior potency, resistance profile, and with improved solubility. Based on this premise, some of our research has been directed towards novel and soluble prodrugs of Amprenavir. We have recently described some of these activities,[2] which culminated in the discovery of phosphate **2**,[3–6] currently in advanced stages of clinical development (Fig. 1).

In the course of our investigations we discovered that the mixed carbonate **3** undergoes O→*N acyloxy* migration to **4**, a nitro derivative of Amprenavir **1** (Fig. 2). We now wish to report on this new class of water-soluble prodrugs, exemplified by **3**,[3–6] which readily converts to drug molecules neutral pH at room temperature.[7]

Although conceptually related prodrug strategy, which utilizes *acyl* transfer has been described,[8,9] the O→*N acyloxy* migration reported herein is novel in the context of prodrugs. Extensive literature searches revealed that reports of intramolecular O→*N acyloxy* migration are rare and usually occur in aromatic systems such as anilines under forcing, non-physiological conditions.[10–13] In spite of this limited precedent, we decided to explore this transformation in the context of Amprenavir prodrugs.

In order to avoid the necessary protection/deprotection of the anilino group in Amprenavir, we based our explorations on the nitro-derivative **3**. Our initial strategy towards **3** involved the synthesis of Boc-protected **7**, which could be obtained from intermediate **5**, previously utilized in syntheses of other Amprenavir analogues,[3–6] and 3-tetrahydrofurane chloroformate. This approach was first evaluated with commercially available methyl and allyl chloroformates. Low to medium yields of respective carbonates **10** and **11** necessitated time-consuming chromatographies,[14] and in order to optimize this chemistry we embarked on an alternative route employing nitrophenyl carbonate **6**. After a considerable experimentation, we developed an efficient synthesis of **6** from **5** and bis (*p*-nitro-phenyl) carbonate in presence of either P4-phosphazene base (61% yield), or lithium hydroxide (80% yield, Fig. 3). Next, target molecule **7**[15] was conveniently synthesized by reacting **6** with (*S*)-(+)-3-hydroxy-tetrahydrofuran in refluxing dioxane. Other carbonates, including those of primary

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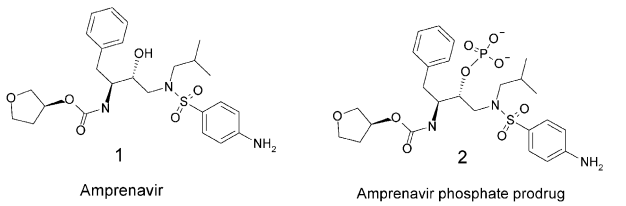


Figure 1. Chemical structures of Amprenavir **1** and its phosphate prodrug **2**.

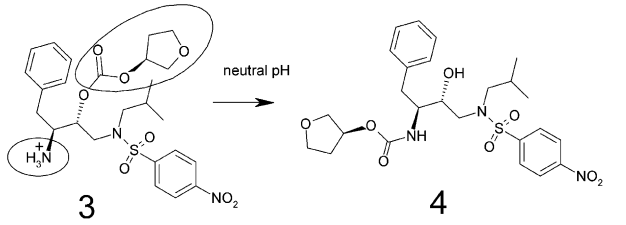


Figure 2. Structures of nitro-Amprenavir **4** and its carbonate prodrug **3**.

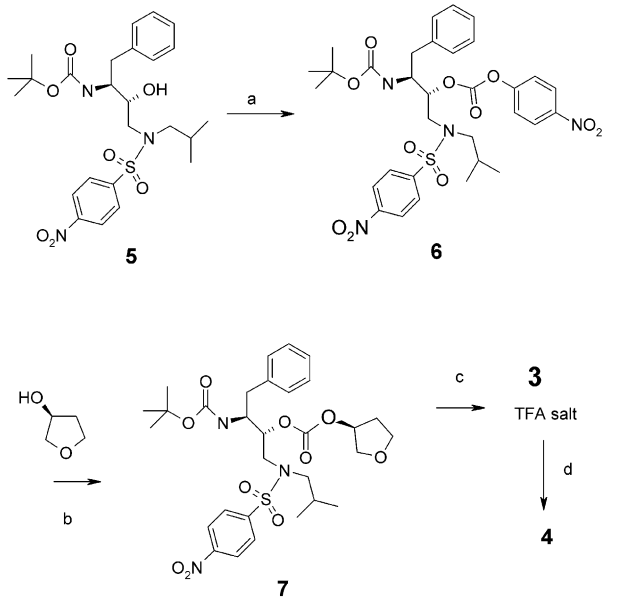


Figure 3. (a) $LiOH{\cdot}H_2O$ in dioxane, bis-(*p*-nitrophenyl) carbonate; (b) dioxane, 3 equiv triethylamine; (c) 50% TFA in DCM; (d) 1:1 (v/v) buffer/THF.

alcohols could also be obtained using similar methodology (not shown). Subsequent TFA-mediated deprotection of Boc carbonate **7** cleanly yielded the TFA salt of **3** (Fig. 3).

We then subjected **3** to simulated physiological conditions [1:1 (v/v) buffer/THF at room temperature] and attempted to induce the O→*N acyloxy* migration. We were pleased to observe a fast conversion of the carbonate to new product, later found identical to the authentic sample of **4**.[3,5,6] We also demonstrated that analogous migration occurred in both methyl (**10**) and allyl (**11**)[14] prodrugs (Fig. 4), which pointed to a general applicability of this approach.

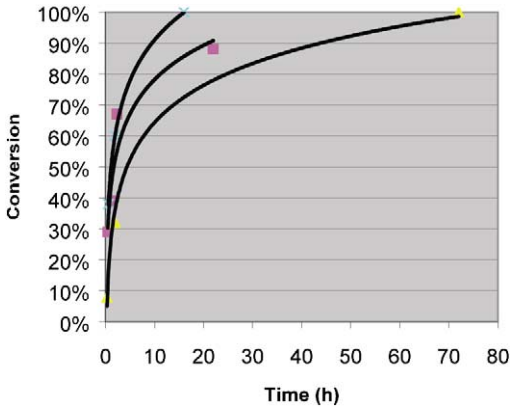
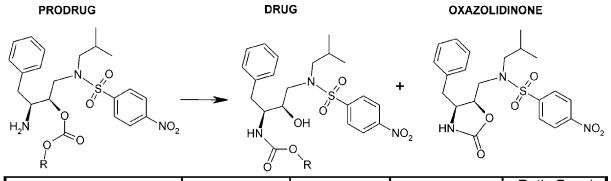


Figure 4. Substituent effect on the kinetics of O→*N acyloxy* migration at pH = 7.0. Left to right, prodrugs **3**, **10** and **11**.



R	Prodrug (compound number)	Drug (compound number)	Oxazolidin-2-one (compound number)	Ratio Drug/ oxazolidin-2-one (by ^{1}H NMR)
Methyl	**10**	**12**	**14**	3
Allyl	**11**	**13**	**15**	11.5
S(+)-3-tetrahydrofurane	**3**	**4**	**16**	2.2

Figure 5. Formation of oxazolidin-2-ones in the O→*N acyloxy* migration.

Detailed analysis of this reaction revealed formation of minor quantity of oxazolidin-2-one, presumably by elimination of alcohol during the acyloxy rearrangement. We further determined ratios of drug to oxazolidin-2-one by ^{1}H NMR (Fig. 5). Allyl prodrug **11**, the slowest converting compound in this set (Fig. 4), gave the highest ratio of drug to oxazolidin-2-one (Fig. 5). We speculated that more stable prodrugs could further reduce or even eliminate the formation of oxazolidin-2-one.

In conclusion, we have developed efficient chemistry towards novel mixed-carbonate prodrugs of the type **3**, and demonstrated that under simulated physiological conditions these undergo swift O→*N acyloxy* migration to carbamate-based protease inhibitors, such as **4**. Such a migration is unprecedented in the context of prodrugs and differs from the more classical hydrolytic approaches to prodrugs, in which the 'pro'-moiety is hydrolized off. In these cases, the 'pro'-moiety is irretrievably lost, resulting in a poor atom economy. In addition, potential toxicity issues of cleaved 'pro'-moieties limit their choices. We believe that the approach described herein addresses some of these drawbacks and that it can be utilized generally as a prodrug approach towards carbamate moiety-bearing drugs.

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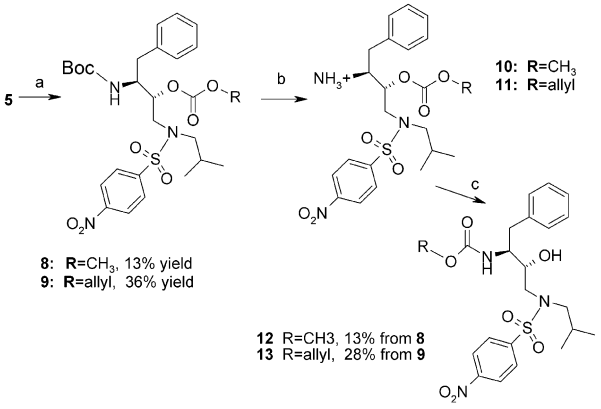
14. 1.



Compound 8: *N*-BuLi (1.0 mL, 2.5 mmol) and methyl chloroformate (0.0849 mL, 1.1 mmol) were added to **5** (0.521 g, 1 mmol) in tetrahydrofuran (5.0 mL) at 0 °C under nitrogen atmosphere. The mixture was gradually warmed to room temperature over 3 h. After quenching with water and extracting with ethyl acetate, the organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography with ethyl acetate/hexane (1:2) to give 75 mg of the desired product **8** (13% yield).

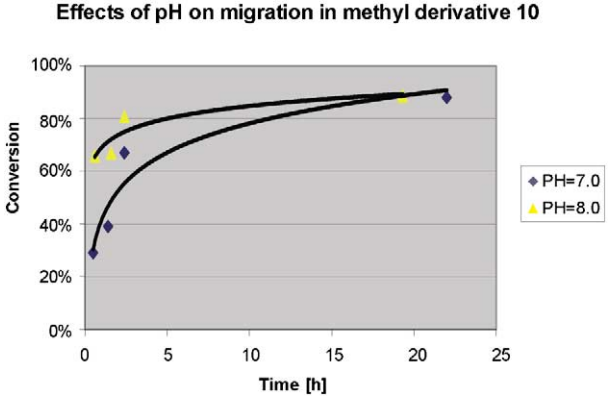
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (2H, d, J = 8.5 Hz), 8.00 (2H, d, J = 8.8 Hz), 7.37–7.21 (5H, m), 5.03 (1H, m), 4.61 (1H, m), 4.07 (1H, m), 3.75 (3H, s), 3.55–3.34 (2H, m), 3.10 (1H, m), 2.94 (2H, m), 2.71 (1H, m), 1.95 (1H, m), 1.30 (9H, s), 0.87 [6H, distorted doublet, J (apparent) = 6.3 Hz]. LRMS: m/z 580.1 (MH^+).

Compound 10: Dichloromethane (2 mL) and trifluoroacetic acid (2 mL) were added to **8** (0.035 g, 0.0604 mmol), and the mixture stirred at room temperature for 20 min.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (2H, d, J = 8.6 Hz), 7.97 (2H, d, J = 8.6 Hz), 7.30 (5H, m), 5.02 (1H, m), 3.80 (1H, m), 3.61 (3H, s), 3.38 (1H, m), 3.26 (1H, m), 2.95 (1H, m), 2.89 (1H, m), 2.79 (2H, m), 1.79 (1H, m), 0.70 (6H, pseudo d).

O→*N* *acyloxy* migration: 1:1 (v/v) mixture of THF and potassium phosphate monobasic–sodium hydroxide buffers (0.05 M, pH 7.0 or 8.0) were used to investigate the O→*N* *migration*. Concentration of prodrug in the buffer was ca. 6 mM. Thus, **10** (0.030 g, 0.0625 mmol) was dissolved in phosphate buffer (pH 7.0) and THF (5 + 5 mL), and the reaction was LC/MS monitored at room temperature. Upon completion, preparative HPLC purification yielded 4 mg of **12**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (2H, d, J = 8.8 Hz), 8.02 (2H, d, J = 8.8 Hz), 7.21-7.11 (5H, m), 7.02 (1H, m), 5.02 (1H, m), 3.60 (1H, m), 3.41 (1H, m), 3.35 (3H, s), 3.02-2.96 (2H, m), 2.91 (1H, m), 2.86 (2H, m), 2.83 (1H, m), 1.91(1H, m), 0.82-0.77 (6H, m). LRMS: m/z 480.1 (MH^+).

As expected, conversion rates generally were faster at pH 8 (as exemplified below).



15. **The synthesis of 6**

Phosphazene method. Bis-(*p*-nitrophenyl) carbonate (13.39 g, 43.56 mmol in 44.62 mL DMF) and phosphazene base P_4-*t*-Bu (10.89 mL, 1 M in hexane, 10.89 mmol) were added to (0.50 g, 0.99 mmol) of **5**. The mixture was stirred at room temperature for approximately 6 h, washed with ethyl acetate, 1 N HCl, brine, and 1 N NaOH sequentially, dried over MgSO4, and concentrated. The residue was purified by silica gel column chromatography with ethyl acetate/hexane (1/2) (61% yield of **6**).

^{1}H NMR: δ 8.41 (2H, d, J = 8.8 Hz), 8.34 (2H, d, J = 9.0 Hz), 8.11 (2H, d, J = 8.8 Hz), 7.47 (2H, d, J = 9.1 Hz), 7.30–7.23 (5H, m), 7.21 (1H, m), 4.94 (1H, m), 4.17 (1H, m), 3.59–3.45 (2H, m), 3.04–2.91 (2H, m), 2.84–2.67 (2H, m), 1.92 (1H, m), 1.29 (9H, s), 0.85 (6H, d, J = 6.1 Hz). LRMS: m/z 687.3 (MH^+).

LiOH method. LiOH monohydrate (0.089 g, 2.2 mmol) was added to (0.10 g, 0.192 mmol) of **5** and (0.64 g, 2.10 mmol) of bis-(*p*-nitrophenyl) carbonate in DMF (1 mL). This mixture was stirred at room temperature for about 2 h, diluted with ethyl acetate, extracted with HCl, NaOH, and brine. Yield 80%.

Compound 7. 150 mg of **6** in 3 mL of anhydrous dioxane was combined with 0.35 mL of (*S*)-(+)-3-hydroxy-tetrahydrofuran and 0.14 mL of triethylamine and refluxed for 48 h (yield quantitative). ES+ 636.2 (M + 1).

^{1}H NMR ($CDCl_3$) δ 8.29 (2H, d), 7.91 (2H, d), 7.22 (5H, m), 5.13 (1H, m), 4.96 (1H, m), 4.52 (1H, d), 4.02 (1H, m), 3.84 (2H, m), 3.44 (1H, m), 3.36 (1H, m), 3.10 (3H, m, overlap), 2.88 (2H, m), 2.64 (1H, m), 2.14 (1H, m), 2.05 (1H, m), 1.84 (1H, m), 1.27 (9H, s), 0.78 (6H, two overl. d).

Compound 3. Obtained by treatment of **7** with 50% TFA/DCM for 30 min and removal of solvents. ^{1}H NMR (methanol-d_4): δ 8.39 (2H, d, J = 8.9 Hz), 8.23 (2H, m), 8.04 (2H, d,

$J = 8.9$ Hz), 7.40–7.29 (5H, m), 5.14 (1H, m), 5.09 (1H, m), 3.84 (1H, m), 3.78 (1H, m), 3.72 (3H, m), 3.46 (1H, m), 3.35 (1H, m), 3.04 (1H, m), 2.93 (1H, m), 2.84 (2H, m), 2.16 (1H, m), 1.90 (1H, m), 1.84 (1H, m), 0.74 (6H, m)

The conversion of **3** to **4** was performed under conditions described for **10**.

Compound 4. ^{1}H NMR: δ 8.38 (2H, d, $J = 8.8$ Hz), 8.05 (2H, d, $J = 8.7$ Hz), 7.33–7.14 (5H, m), 7.12 (1H, m), 4.95 (1H, m), 3.70 (2H, m), 3.60 (1H, m), 3.51 (1H, m), 3.52 (1H, m), 3.39 (1H, m), 3.36 (1H, m), 3.14 (1H, m), 3.07 (1H, m), 2.94 (2H, m), 2.47 (1H, m), 2.05 (1H, m), 1.97 (1H, m), 1.76 (1H, m), 0.84 (6H, m).