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Synthesis and evaluation of carbamoylmethylene linked prodrugs of BMS-582949, a clinical p38α inhibitor

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ABSTRACT

A series of carbamoylmethylene linked prodrugs of 1 (BMS-582949), a clinical p38α inhibitor, were synthesized and evaluated. Though the phosphoryloxymethylene carbamates (**3**, **4**, and **5**) and α-aminoacyloxymethylene carbamates (**22**, **23**, and **26**) were found unstable at neutral pH values, fumaric acid derived acyloxymethylene carbamates (**2**, **28**, and **31**) were highly stable under both acidic and neutral conditions. Prodrugs **2** and **31** were also highly soluble at both acidic and neutral pH values. At a solution dose of 14.2 mpk (equivalent to 10 mpk of **1**), **2** gave essentially the same exposure of **1** compared to dosing 10 mpk of **1** itself. At a suspension dose of 142 mpk (equivalent to 100 mpk of **1**), **2** demonstrated that it could overcome the solubility issue associated with **1** and provide a much higher exposure of **1**. To our knowledge, the unique type of prodrugs like **2**, **28**, and **31** was not reported in the past and could represent a novel prodrug approach for secondary amides, a class of molecules frequently identified as drug candidates.

We have previously disclosed **1** (BMS-582949) (Fig. 1) as a highly selective p38α inhibitor that advanced to Phase II clinical trials for the treatment of rheumatoid arthritis (RA).[1] An important consideration for the development of **1** is its pH dependent solubility, which was determined to be 0.280 mg/mL at pH 1.2 and 0.003 mg/mL at pH 6.5. This presents a potential limit to its usage in RA patients, who are often prescribed drugs that increase stomach pH, including H_2 blockers such as famotidine.[2,3] Systemic exposure of **1** can be expected to be significantly lower in patients using such co-medications. While formulation studies were under way, we speculated that this potential issue could be addressed by implementing a prodrug strategy. To avoid additional development complications, we set a high bar for the prodrug criteria. Specifically, in addition to improving the solubility profile, the prodrug needed to be chemically stable between pH 1 and pH 7; completely bio-converted to **1** before or during absorption; have parent drug exposure upon prodrug administration comparable to or better than what was observed with **1** itself; and lastly, the safety profiles of its in vivo by-products should be known or predictable. Our investigation yielded prodrug **2** that met all the afore-mentioned criteria.

The parent compound **1** contains two amide NH groups and one diaryl NH that can be considered as logical handles to install promoieties. However, prodrugs derived from amide NH or diaryl NH are not well-documented in the literature except for some pioneering studies. These studies include investigations of Mannich base, sulfenamide, and other typed prodrugs,[4,5] which were not attractive to us according to our specific prodrug criteria for **1**. On the other hand, it was noted that prodrugs of aliphatic amines were more often seen in literature.[6] One of the prodrug approaches for amines is the conversion of the parent amine into acyloxymethylene or phosphoryloxymethylene carbamates. For example, hydrophobic acyloxymethylene carbamates of pseudomycin analogs were reported to improve toxicity profiles,[7] while phosphoryloxymethylene carbamates of paclitaxels were synthesized to increase aqueous solubility.[8] The design of these types of amine prodrugs were based on the rationale that acyloxymethylene and phosphoryloxymethylene carbamates would be hydrolyzed in vivo by esterases and phosphatases, respectively, to form a hydroxymethylene carbamate as the immediate intermediate that would be chemically unstable and spontaneously decompose to the parent amine, formaldehyde, and carbon dioxide.[9,10] Any concerns about the generation of formaldehyde during the bio-conversion of these types of prodrug were lessened by the consideration that humans produce and/or may be exposed to 31–59 g of formaldehyde daily and that several prodrugs that release formaldehyde were approved by regulatory agencies.[11]

Inspired by the literature precedents of carbamoylmethylene linked prodrugs of aliphatic amines, we wondered if we could

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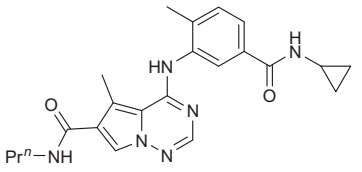

1 (BMS-582949)

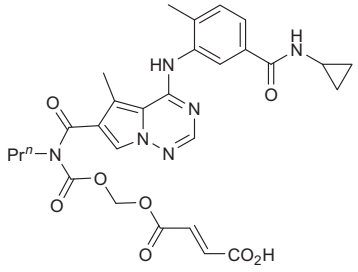

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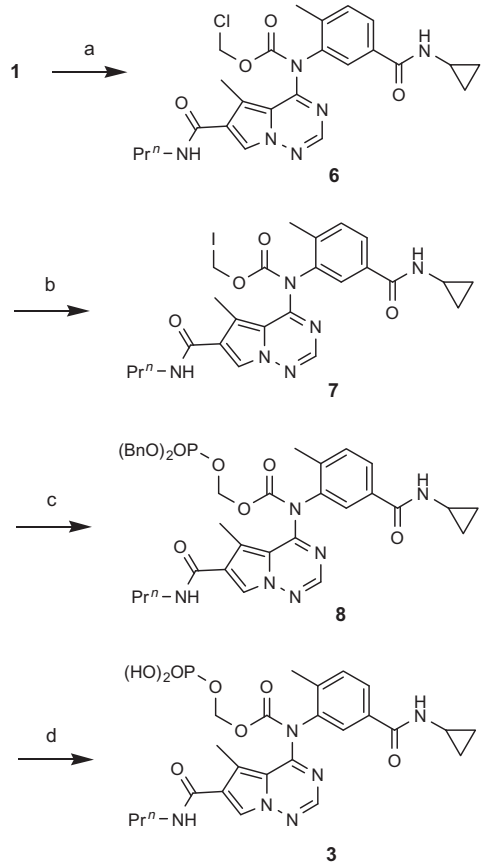
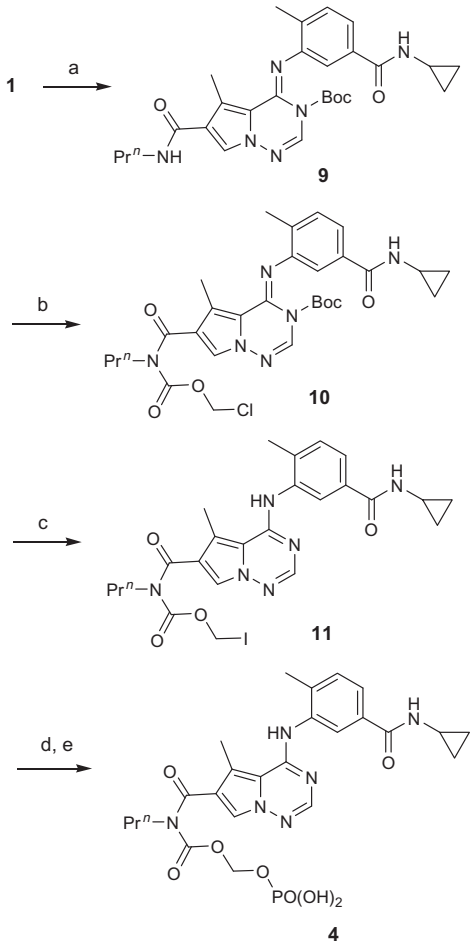
Figure 1. Clinical p38α inhibitor **1** and its prodrug **2**.

prepare any carbamoylmethylene linked phosphate prodrugs of **1** through any of its NH groups. To our satisfaction, it was found that all three possible isomeric prodrugs, **3**, **4**, and **5** (Schemes 1–3, respectively), could be synthesized. Two of the three were derived directly from **1** by taking advantage of the pK_a differences among the three NH groups. As shown in Scheme 1, treatment of **1** with chloromethyl chloroformate in the presence of pyridine provided chloromethyl carbamate **6**. The latter was converted to iodomethyl



Scheme 1. Reagents and conditions: (a) chloromethyl chloroformate, pyridine, CH_2Cl_2, 50 °C, 63% yield; (b) NaI, acetone, reflux; (c) silver dibenzyl phosphate, toluene, reflux, 51% yield over two steps; (d) H_2, 10% Pd/C, rt, 100% yield.

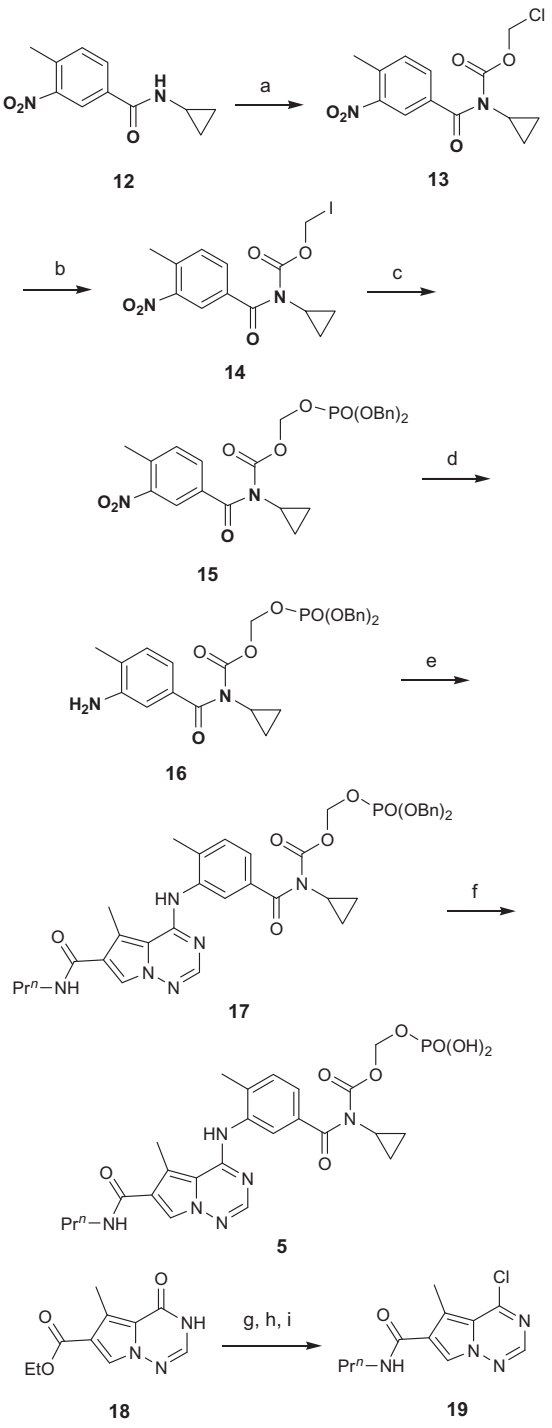


Scheme 2. Reagents and conditions: (a) di-*tert*-butyl dicarbonate, DMAP, DMF, 60 °C, 70% yield; (b) 2 equiv lithium bis(trimethylsilyl)amide, THF, −45 °C, then 1 equiv chloromethyl chloroformate, −45 °C to rt, 60% yield; (c) NaI, acetone, reflux, 61%; (d) silver dibenzyl phosphate, toluene, reflux, 26% yield over two steps; (e) H_2, 10% Pd/C, rt, 90% yield.

carbamate **7** with sodium iodide. Heating **7** with silver dibenzyl phosphate supplied **8**, which was hydrogenated to form the desired prodrug **3**.

For the preparation of **4** (Scheme 2), **1** was reacted with di-*tert*-butyl dicarbonate in the presence of 4-dimethylaminopyridine (DMAP) to afford **9**. Interestingly, it was the pyrrolotriazine ring nitrogen that was acylated instead of the anilino nitrogen. The structure of **9** was revealed by X-ray crystallography (Fig. 2).[12] Treatment of **9** with 2 equiv of lithium bis(trimethylsilyl)amide, followed by 1 equiv of chloromethyl chloroformate, led to the formation of chloromethyl carbamate **10** regioselectively. The regioselectivity of this carbamoylation can be rationalized in that the *N*-propyl amide NH is most likely less acidic than the *N*-cyclopropyl benzamide NH, and therefore its conjugated base *N*-propyl amide nitrogen anion is more basic and nucleophilic. When **10** was refluxed with sodium iodide in acetone to convert the chloromethyl carbamate to a iodomethyl carbamate, it was pleasantly found that the Boc group was cleaved under the same conditions, generating **11** from **10** in one pot. Prodrug **4** was then obtained from **11** in a similar manner as in the preparation of **3** from **7**.

To synthesize **5** (Scheme 3), *N*-cyclopropyl benzamide **12** was deprotonated with lithium bis(trimethylsilyl)amide and then exposed to chloromethyl chloroformate to furnish **13**. Chloromethyl carbamate **13** was transformed into carbamoylmethylenephosphate **15** by sodium iodide, followed by silver dibenzyl phosphate.



Scheme 3. Reagents and conditions: (a) bis(trimethylsilyl)amide, THF, −78 °C, then chloromethyl chloroformate, 0 °C to rt, 72% yield; (b) NaI, acetone, reflux, 90%; (c) silver dibenzyl phosphate, toluene, reflux, 83% yield; (d) Zn dust, NH_4Cl, 1:1 MeOH/THF, rt, 100% yield; (e) **18**, DMF, rt, 43% yield; (f) H_2, 10% Pd/C, rt, 60% yield; (g) 1 N NaOH, MeOH, reflux, 72% yield; (h) *n*-$PrNH_2$, BOP, 1-methylmorpholine; (i) $POCl_3$, *i*-Pr_2NEt, toluene, reflux, 61% yield.

Reduction of **15** with zinc dust in the presence of ammonium chloride gave **16**, which was then reacted with chloropyrrolotriazine **19** to form **17**. Intermediate **19** was prepared from ethyl 5-methyl-4-oxo-3,4-dihydropyrrolo[1,2-*f*][1,2,4]triazine-6-carboxylate (**18**)[13] in three steps: hydrolysis of the ester group, amide formation from the resultant acid, and the treatment of $POCl_3$. Hydrogenation of **17** completed the synthesis of prodrug **5**.

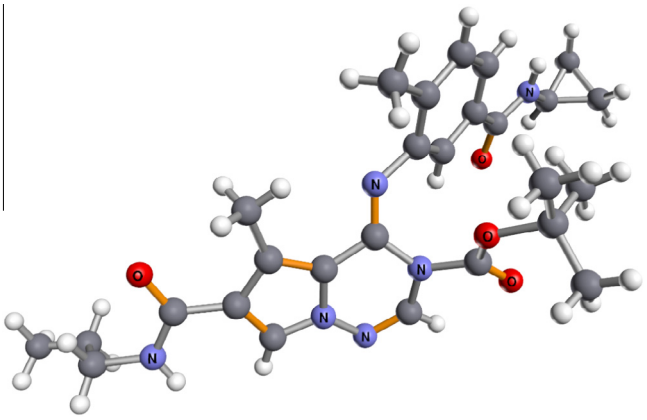


Figure 2. X-ray crystal structure of **9**.

Table 1
Stability profiles of **3**, **4**, and **5**

	Stability at 37 °C		
	3	**4**	**5**
$T_{1/2}$ at pH 1.0 (h)	8.5	26.8	20.5
$T_{1/2}$ at pH 7.4 (h)	<0.25	<0.25	<0.25

With **3**, **4**, and **5** in hand, we began our prodrug evaluations with solution stability tests, the results of which are shown in Table 1. Compounds **3**, **4**, and **5** were stable at pH 1.0 and 37 °C with their half lives being 8.5, 26.8, and 20.5 h, respectively. However, they were all unstable under more physiologically relevant conditions (pH 7.4, 37 °C), as their half lives were determined to be less than 0.25 h. It was noted that the stability profiles displayed by **3**, **4**, and **5** at pH 7.4 were very different from that of an aliphatic amine derived phosphoryloxymethylene carbamate **20**, the half life of which was reported to be 48 days at pH 7.5 and 25 °C (Fig. 3).[10] Instead, their stabilities were more similar to what was observed for phosphoryloxymethyl carbonate **21** ($T_{1/2}$ = 58 min at pH 7.5 and 25 °C). The short half life for **21** at neutral pH was attributed to an intramolecular phosphate-assisted hydrolysis or an intramolecular nucleophilic reaction of the phosphate oxygen anion to the carbonate carbonyl, which is more electrophilic than the carbamate carbonyl in **20**. This proposal can be used to explain the instability of **3**, **4**, and **5** under similar conditions. Though they are carbamates, the carbamate carbonyls are highly activated by either *N*,*N*-diaryl or *N*-acyl groups.

Attempting to obtain prodrugs of **1** with improved stability, we synthesized α-amino aminoacyloxymethylene carbamates **22**, **23**, and **26** (Scheme 4). Reaction of intermediate **6** and silver salt of (*S*)-2-(benzyloxycarbonylamino)propanoic acid (**27**) in refluxing toluene, followed by hydrogenation with 10% Pd/C in the presence of 1 N hydrochloric acid, provided **22**. In a similar manner, **23** was synthesized from iodide **11**. The silver salt **27** was prepared by treating (*S*)-2-(benzyloxycarbonylamino)propanoic acid with so-

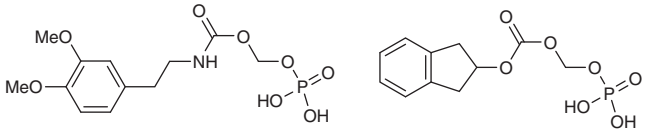
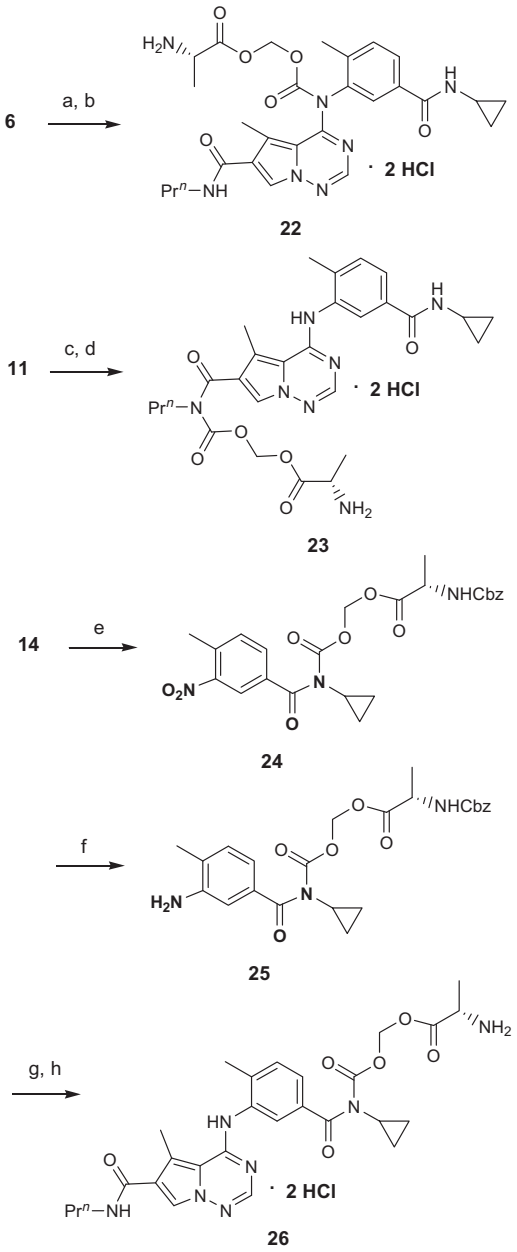


Figure 3. Literature examples of phosphoryloxymethylene carbamate and phosphoryloxymethylene carbonate with their stability profiles[10].



Scheme 4. Reagents and conditions: (a) silver salt of (*S*)-2-(benzyloxycarbonylamino)propanoic acid (**27**), toluene, reflux, 39% yield; (b) H_2, 10% Pd/C, MeOH, 1 N HCl, 80% yield; (c) **27**, toluene, reflux, 19% yield; (d) H_2, 10% Pd/C, MeOH, 1 N HCl, 91% yield; (e) **27**, toluene, reflux, 66% yield; (f) Zn dust, NH_4Cl, 1:1 MeOH/THF, rt, 39% yield; (g) **19**, DMF, rt, 76% yield; (h) H_2, 10% Pd/C, MeOH, 1 N HCl, 67% yield.

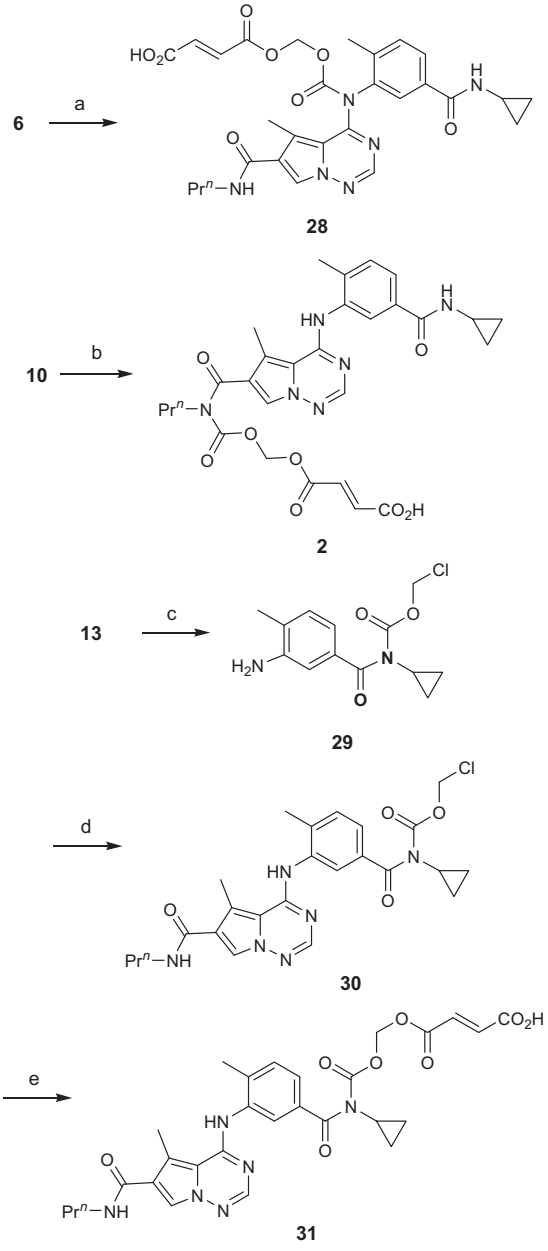
dium hydroxide and silver nitrate sequentially. Heating **14** with **27** gave rise to **24**, which was reduced with zinc dust to aniline **25**. Then, **26** was obtained by reacting **25** with chloropyrrolotriazine **19** in DMF, followed by hydrogenation to remove the Cbz protection group.

Unfortunately, like phosphoryloxymethylene carbamates **3**, **4**, **5**, the α-amino aminoacyloxymethylene carbamates **22**, **23**, and **26** were all unstable at pH 7.4 and 37 °C with their half lives being less than 0.25 h (Table 2). Also, they were only moderately stable at pH 1.0 ($T_{1/2}$ = 3.0–3.2 h). These stability results, though disappointing, were not too surprising considering that an amine is a good nucleophile at neutral pH values and an intramolecular nucleophilic attack at the highly activated carbamoyl carbonyl by the amino may easily occur.

Table 2
Stability profiles of **22**, **23**, and **26**

	Stability at 37 °C		
	22	**23**	**26**
$T_{1/2}$ at pH 1.0 (h)	3.2	3.2	3.0
$T_{1/2}$ at pH 7.4 (h)	<0.25	<0.25	<0.25

Ultimately, fumaric acid derived acyloxymethylene carbamates **28**, **2**, and **31** were designed, with the idea that the *trans* configuration of the fumarate would prevent the promoiety from any intramolecular cleavage. Fumaric acid, a likely metabolic by-product of these prodrugs, is expected to to safe, as it is among those acids approved by FDA to form pharmaceutic salts.[14] Thus, intermediates **6**, **10**, and **30** were reacted with monosodium salt of fumaric acid to provide **28**, **2**, and **31**, respectively (Scheme 5).



Scheme 5. Reagents and conditions: (a) monosodium salt of fumaric acid, 115 °C, 5.5 h, DMF, 20% yield; (b) monosodium salt of fumaric acid, 105 °C, 20 h, DMF, 8.5% yield; (c) Zn dust, NH_4Cl, 1:1 MeOH/THF, rt, 7 h, 94% yield; (d) **19**, rt, DMF, 20 h; (e) monosodium salt of umaric acid, 105 °C, DMF, 5 h, 17% yield for two steps.

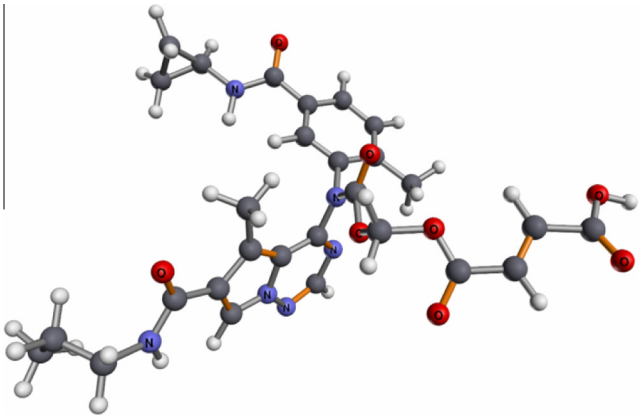


Figure 4. X-ray crystal structure of **28**.

Intermediate **30** was prepared by reduction of **13** with zinc dust in the presence of ammonium chloride, followed by a subsquent reaction with chloropyrrolotriazine **19**. The structure of **28** was confirmed by X-ray crystallography (Fig. 4),[15] which also provided indirect confirmation of the structure of carbamate **6**.

We were delighted to find that prodrugs **28**, **2**, and **31** were highly stable not only under acidic conditions but also under neutral conditions. Their half lives ranged from 34.5 to 129 h (at pH 1.0) and from 14.6 to 21.8 h (at pH 7.4) (Table 3). Among the three, **28** showed the highest stability at pH 1.0 but the lowest at pH 7.4. Between prodrugs **2** and **31**, **2** was noticeably more stable than **31** at both acidic and neutral pH values.

After the chemical stability goal was achieved with **28**, **2**, and **31**, we proceeded to evaluate the aqueous solubility for these three prodrugs. At pH 6.5, all the three were highly soluble (Table 4). Their aqueous solubilities were measured to be 1.154, 0.883, and 1.570 mg/mL, dramatically better than that of the parent drug **1** (0.003 mg/mL) at the same pH value. However, at pH 1.0, the solubilities were very different among the three. Prodrug **28** was poorly soluble (0.008 mg/mL). Prodrug **2** exhibited a solubility of 1.178 mg/mL, which was seven fold higher compared to what was observed for **31** (0.166 mg/mL). For **2**, we also determined its solubility at pH 3.1 and 4.6 to be 0.105 and 0.367 mg/mL, respectively. The decrease in solubility at these pH values is most likely due to the formation of zwitter-ion.

With the measurement of the chemical stability and aqueous solubility, prodrug **2** emerged as a leading candidate. Evaluation of the bio-conversion of **2** into its parent drug **1** was then conducted by oral dosing to rats and then determining the exposure of **1** in plasma. As shown in Table 5, after a PEG solution dose of 14.2 mpk (equivalent to 10 mpk of **1**), prodrug **2** gave almost identical exposure of **1** compared to what was observed with 10 mpk of **1** itself. The AUC_{0-24h} of **1** after dosing **2** was 52.0 μM h versus 53.3 μM h after dosing **1**. The C_{max} values were also comparable (4.6 vs 6.5 μM). More impressively, after a higher dose of 142 mpk (equivalent to 100 mpk of **1**) with a methocel suspension (Table 6), prodrug **2** provided an AUC_{0-24h} of 213.9 μM h of **1**, which is 38-fold higher than what was obtained by dosing 100 mpk of **1** itself. The C_{max} of **1** was also much higher after dosing **2** compared to dosing **1** itself (21.1 vs 0.8 μM) due to solubility-limited absorption of the parent. It was noticed that in both the solution and suspension doses, the T_{max} values after the prodrug dosing were 3–4 h longer than the parent drug dosing. This may suggest that the prodrug bio-conversion rate is lower than the parent drug absorption rate. In both studies, prodrug **2** was not detected in plasma.

In summary, to address the issue of pH dependent solubility and absorption associated with the clinical p38α inhibitor **1**, a number of carbamoylmethylene linked prodrugs of **1** were synthesized and evaluated. The phosphoryloxymethylene carbamates (**3**, **4**, and **5**) and α-aminoacyloxymethylene carbamates (**22**, **23**, and **26**) were unstable at neutral pH values. However, fumaric acid derived acyloxymethylene carbamates (**28**, **2**, and **31**) were highly stable under both acidic and neutral conditions. Prodrugs **2** and **30** also dispayed good aqueous solubility at both acidic and neutral pH values. Prodrug **2** was evaluated in vivo for its bio-conversion to **1**. It gave essentially the same exposure of **1** at a solution dose of 14.2 mpk (equivalent to 10 mpk of **1**). At a suspension dose of 142 mpk (equivalent to 100 mpk of **1**), **2** provided a much higher exposure of **1** compared to what was obtained by dosing **1** itself. To our knowledge, the carbamoylmethylene linked prodrugs such as **28**, **2**, and **31** have not been previously reported and represent a novel prodrug approach for secondary amides, a class of molecules frequently identified as drug candidates.

Table 3
Stability profiles of **28**, **2**, and **31**

	Stability at 37 °C		
	28	**2**	**31**
$T_{1/2}$ at pH 1.0 (h)	129	79.4	34.5
$T_{1/2}$ at pH 7.4 (h)	14.6	38.5	21.8

Table 4
Solubility profiles of **28**, **1**, and **31**

	Aqueous stability (mg/mL)		
	28	**2**	**31**
pH 1.0	0.008	1.178	0.166
pH 3.1		0.105	
pH 4.6		0.367	
pH 6.5	1.154	0.883	1.570

Table 5
Exposure of **1** after a solution dose of prodrug **2** and parent drug **1**

	Compd	
	2	**1**
Dose[a] (mpk)	14.2 (corresponding to 10 mpk of **1**)	10
T_{max} (h)	4	1
C_{max} (μM)	4.6	6.5
AUC_{0-24h} (μM h)	52.0	53.3

[a] Vehicle: 1:1 PEG 300/water.

Table 6
Exposure of **1** after a suspension dose of prodrug **2** and parent drug **1**

Compd	**2**	**1**
Dose[a] (mpk)	142 (corresponding to 100 mpk of **1**)	100
T_{max} (h)	6	1.7
C_{max} (μM)	21.1	0.8
AUC_{0-24h} (μM h)	213.9	5.6

[a] Vehicle: 0.75% methocel suspension.

References and notes

1. Liu, C.; Lin, J.; Wrobleski, S. T.; Lin, S.; Hynes, J., Jr.; Wu, H.; Dyckman, A. J.; Li, T.; Wityak, J.; Gillooly, K. M.; Pitt, S.; Shen, D. R.; Zhang, R. F.; McIntyre, K. W.; Salter-Cid, L.; Shuster, D. J.; Zhang, H.; Marathe, P. H.; Doweyko, A. M.; Sack, J. S.; McKinnon, M.; Dodd, J. H.; Barrish, J. C.; Schieven, G. L.; Leftheris, K. *J. Med. Chem.* **2010**, *53*, 6629.

2. Taha, A. S.; Hudson, N.; Hawkey, C. J.; Swannell, A. J.; Trye, P. N.; Cottrell, J.; Mann, S. G.; Simon, T. J.; Sturrock, R. D.; Russell, R. I. *N. Eng. J. Med.* **1996**, *334*, 1435.
3. Schiff, M.; Peura, D. *Expert Rev. Gastroenterol. Hepatol.* **2012**, *6*, 25.
4. For a recent review Guarino, V. R.; Stella, V. J. *Biotechnol. Pharm. Aspects* **2007**, *5*, 133.
5. Nti-Addae, K. W.; Stella, V. J. J. *J. Pharm. Sci.* **2011**, *100*, 1001.
6. For recent reviews (a) Simplício, A. L.; Clancy, J. M.; Gilmer, J. F. *Molecules* **2008**, *13*, 519; (b) Krise, J. P.; Oliyai, R. *Biotechnol. Pharm. Aspects* **2007**, *5*, 101.
7. Sun, X.; Zeckner, D. J.; Zhang, Y.-Z.; Sachs, R. K.; Current, W. L.; Rodriguez, M.; Chen, S.-H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1881.
8. Scola, P. M.; Kadow, J. F.; Vyas, D. M. EP 747,385; **1996**.
9. Alexander, J.; Cargill, R.; Michelson, S. R.; Schwamt, H. *J. Med. Chem.* **1988**, *31*, 318.
10. Safadi, M.; Oliyai, R.; Stella, V. J. *Pharm. Res.* **1993**, *10*, 1350.
11. Dhareshwar, S. S.; Stella, V. J. *J. Pharm. Sci.* **2008**, *97*, 4184.
12. CCDC deposition number: 910691.
13. Hynes, J., Jr.; Dyckman, A. J.; Lin, S.; Wrobleski, S.; Wu, H.; Gillooly, K. M.; Kanner, S. B.; Lonial, H.; Loo, D.; McIntyre, K. W.; Pitt, S.; Shen, D. R.; Shuster, D. J.; Yang, X.; Zhang, R.; Behnia, K.; Zhang, H.; Marathe, P. H.; Doweyko, A. M.; Tokarski, J. S.; Sack, J. S.; Pokross, M.; Kiefer, S. E.; Newitt, J. A.; Barrish, J. C.; Dodd, J. H.; Schieven, G. L.; Leftheris, K. *J. Med. Chem.* **2008**, *51*, 4.
14. Berge, S. M.; Bighley, L. D.; Monkhouse, D. C. *J. Pharm. Sci.* **1977**, *66*, 1.
15. CCDC deposition number: 910690.