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Intramolecular Transamidation of β-Lactams as a Means for the Enzymatic Control of Ring Opening: Effect of Substituents on the Rate of Reaction

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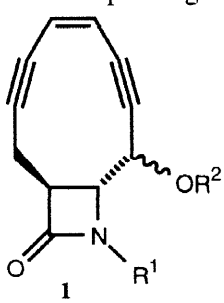
Abstract: A series of simple monocyclic β-lactams bearing side-chains, containing amino groups, have been synthesized, and the rate of their intramolecular transamidation studied. Protection of the amino group with an enzymatically cleavable group, allows us to selectively control the ring enlargement process.

We have recently reported the rational design and preparation of "lactendiynes", a new class of fully synthetic enediyne derivatives, characterized by the fusion of a 10-membered enediynic ring with a β-lactam nucleus.[1] Particularly interesting are compounds of general formula **1**, which are completely stable, while undergoing fast cycloaromatization upon opening of the β-lactam ring. Thus these substances seem promising as stable prodrugs to be selectively activated *in vivo*. In order to allow their employment in selective prodrug activation in tumor cells[2] or in strategies like ADEPT (Antibody Directed Enzyme Prodrug Therapy),[3] or GDEPT (Gene Directed Enzyme Prodrug Therapy),[4] it is however necessary to find a way to trigger the opening of the azetidinone ring with a suitable enzyme. This could be done, for example, by appending, to the lactendiyne nucleus, a handle provided with a nucleophilic moiety capable of effecting intramolecular cleavage of the β-lactam. This moiety could be then protected with an enzymatically removable group.[2,5]



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However, at the outset of this work very little was known on the intramolecular nucleophilic opening of simple monocyclic β-lactams.[6] For this reason, in order to help the rational design of new modified, selectively activated "lactendiynes", we decided to explore, on model compounds, the feasibility of such a reaction at reasonable temperatures, with the goal of also finding a qualitative correlation between substituents on the ring and ease of reaction.

As a nucleophilic group we selected the amino function,[7] which was appended to simple β-lactams at positions 1 and 3[8] through a 2 or 3 atom spacer (Scheme 1).[9] In this way the transition states of the transamidation reaction should be always 5- or 6-membered. The substituent at position 4 was chosen in order to be as similar as possible as the one present in lactendiynes. Finally, the relative configuration of C-3 and C-4 stereogenic centers was always *cis* in our models, since in the final modified lactendiynes, the *trans* position at C-3 will be necessarily occupied by the enediynic ring.

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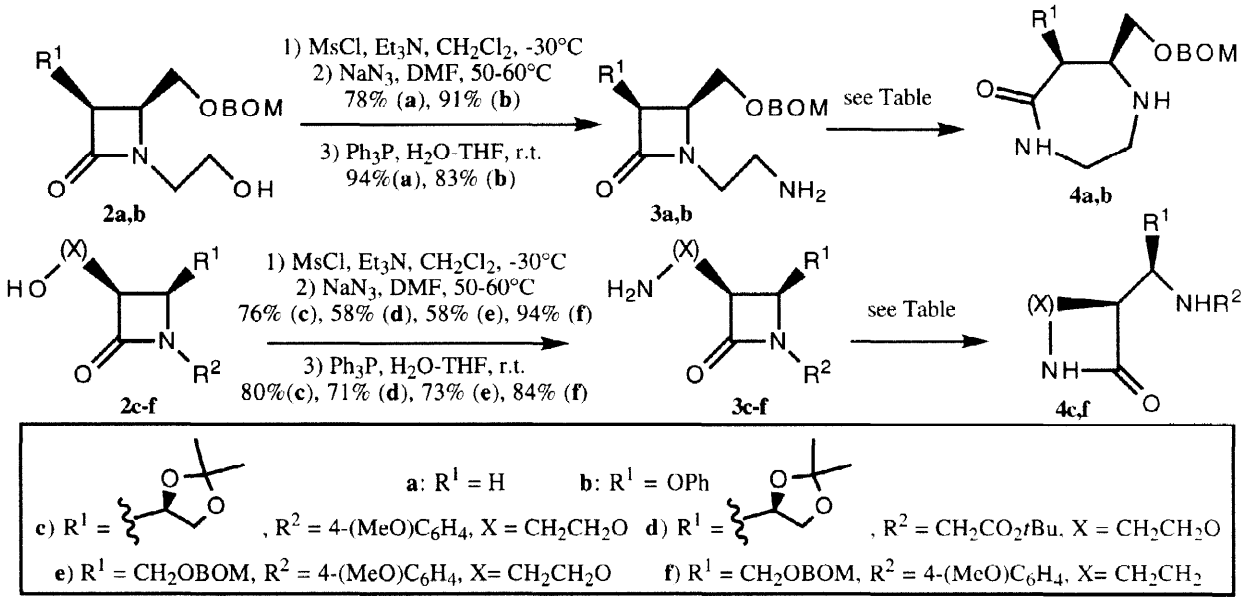
Scheme 1



The general strategy employed for the preparation of amines of general formula **3** encompassed the synthesis of the corresponding alcohols **2**, and their conversion, through azide displacement of the mesylate followed by reduction,[10] into the free amine. While alcohol **2a** could be easily obtained from an intermediate of the previously reported synthesis of lactendiynes,[2b,11] the synthesis of compounds **2b-f** was not an easy task. The preparations[11] all started with a Staudinger condensation of suitable acyl chloride and imine and required careful optimization of both the β-lactam formation and of subsequent transformations. These syntheses will be fully discussed in a forthcoming full paper.

The half life of amines **3a-f** was then examined in two type of solvents: 96% ethanol as a prototype for protic solvents, and 1,2-dichloroethane as an example of an aprotic solvent of low polarity. The results are listed in the Table. In all cases the reactions could be driven to completion after an appropriate time, and thus it appears that, as expected, the equilibrium of the transamidation is shifted to the right.

The dependence of reactivity on structure was first examined in 96% ethanol as solvent. Comparison of entries 1 and 2 indicates that a phenoxy group has a remarkable activating effect on the reaction, making compound **3b** about 10 times more reactive than unsubstituted **3a**. In view of this observation we expected an even higher reactivity of azetidinones **3c,e**, where not only an oxygen atom is present at position 3, but the nitrogen bears an aryl group, which in principle would make the lactam carbonyl more electrophilic. However this was not the case (entries 3,5). The effect of the bulkiness of substituent at C-4 seems negligible. The substitution of anisyl group with a carboxymethyl (entry 4) produced a 6 fold increase of reactivity, which remained however inferior to that of **3b**. Arguing that the lower than expected reactivity of these substrates could be due to the depression of nucleophilicity of the amine caused by the β-oxygen, or to the fact that the transition state for **3c,d,e** is six-membered instead than 5-membered as for **3a,b**, we prepared also amine **3f**, which is characterized by a shorter, non-oxygenated side-chain. However its reactivity in ethanol was even lower than those of **3c,d,e**.

We also performed some preliminary experiments on the influence of concentration and additives on the reaction rate. While, for **3a**, concentration seems unimportant (entry 7), we observed, for both **3a** and **3b** a dramatic effect of added buffer (see entries 8-11). Surprisingly, on amine **3c**, the effect of similar additives was very small.

Table: Intramolecular Transamidation of Amino-substituted β-Lactams 3						
Entry	**Starting Amine**	**Solvent**	**Additives**	**Conc. (M)**	**Temp.**	$t_{1/2}$[a]
1	**3a**	96% EtOH	none	$1.1 \cdot 10^{-2}$	60°C	136 h
2	**3b**	96% EtOH	none	$1.1 \cdot 10^{-2}$	40°C	50 h
3	**3c**	96% EtOH	none	$1.1 \cdot 10^{-2}$	60°C	130 h
4	**3d**	96% EtOH	none	$1.1 \cdot 10^{-2}$	60°C	22 h
5	**3e**	96% EtOH	none	$1.1 \cdot 10^{-2}$	60°C	120 h
6	**3f**	96% EtOH	none	$1.1 \cdot 10^{-2}$	60°C	204 h
7	**3a**	96% EtOH	none	10^{-1}	60°C	130 h
8	**3a**	96% EtOH	0,1 M Et_3N	$1.1 \cdot 10^{-2}$	60°C	173 h
9	**3a**	96% EtOH	0,1 M Et_3N / 0.05M AcOH	$1.1 \cdot 10^{-2}$	60°C	≈ 8 h
10	**3a**	96% EtOH	0,1 M Et_3N / 0.05 M AcOH	$1.1 \cdot 10^{-2}$	40°C	33 h
11	**3b**	96% EtOH	0,1 M Et_3N / 0.05 M AcOH	$1.1 \cdot 10^{-2}$	40°C	≈ 3 h
12	**3c**	96% EtOH	0,17 M Et_3N/ 0.085 M HCl	$1.1 \cdot 10^{-2}$	60°C	120 h
13	**3c**	96% EtOH	0,17 M morpholine/ 0.085 M HCl	$1.1 \cdot 10^{-2}$	60°C	150 h
14	**3a**	CH_2ClCH_2Cl	none	$1.1 \cdot 10^{-2}$	60°C	35 h
15	**3a**	CH_2ClCH_2Cl	none	$7.3 \cdot 10^{-2}$	60°C	34 h
16	**3b**	$CDCl_3$	none	$5 \cdot 10^{-2}$	30°C	18 h
17	**3d**	CH_2ClCH_2Cl	none	$1.1 \cdot 10^{-2}$	60°C	60 h[b]
18	**3e**	CH_2ClCH_2Cl	none	$1.1 \cdot 10^{-2}$	60°C	100 h
19	**3f**	CH_2ClCH_2Cl	none	$1.1 \cdot 10^{-2}$	60°C	50 h[c]

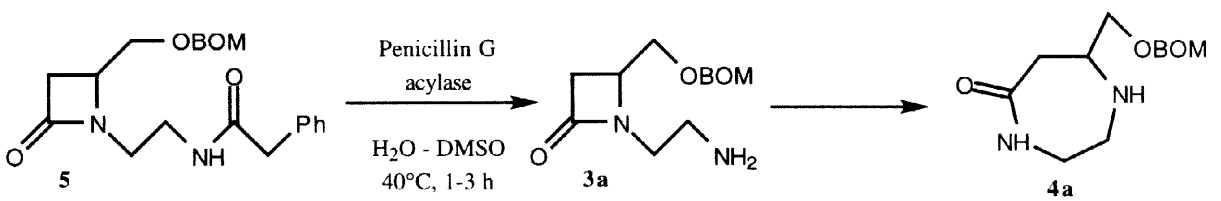
[a] $t_{1/2}$ means the time after which 50% of the starting amine has been converted into the isomerized ε, δ, or γ lactam. It was measured by taking aliquots of the reaction mixture at intervals and examining them at ^1H n.m.r. [b] A small percentage of by-product, deriving from attack of **2d** on CH_2ClCH_2Cl was detected. [c] The main product was in this case the one deriving from attack of **2f** to CH_2ClCH_2Cl.

The results of transamidation in an aprotic solvent like CH_2ClCH_2Cl were in some cases parallel to those in EtOH (compare for example entries 1,2 with 14 and 16). A generally higher reactivity was though observed (with the remarkable exception of **3e**).

In conclusion, although the mechanism of these transamidation reactions appears to be, at first sight, quite complex, and surely merits further investigation, the collected data allowed us to prove that the possibility of intramolecular opening of a β-lactam to give a larger ring at physiological temperatures is real. The study permitted us to select the type of substituents which seem more suited for incorporation in the lactendiynes structure. Studies toward this goal are in progress.

In the meantime we have performed preliminary tests on the possibility of blocking the reactive amino group with an enzymatically cleavable protecting group. Thus amine **3a** (Scheme 2) was acylated with phenylacetyl chloride, and the resulting amide **5** subjected to reaction with penicillin G acylase.[15] Reaction took place smoothly to give back **3a**, which, under the same conditions, was slowly converted to the ε-lactam **4a**.

Scheme 2



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7. Other functional groups, for example the hydroxy group, were anticipated to be not nucleophilic enough, while the thiolate group was more problematic to study on a small scale because of its tendency to react with air to give disulfides.
8. Side chains at position 4 were not included in this study for two reasons: **a**) stereoselective synthesis of *trans* lactendiynes disubstituted at position 4 of the β-lactam was expected to be problematic. **b**) The transition state for transamidation reaction would be in this case a bridged bicyclic ring instead than a *ortho*-fused byciclic ring and thus the reaction was foreseen to be more difficult.
9. All compounds quoted in this communication were fully characterized by ^{1}H n.m.r., I.r., ^{13}C n.m.r. (in most cases), GC-MS (when feasible), and were all racemic.
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11. *Preparation of alcohols* ***2a,f***: **2a**: From 1-(*tert*-butyldimethylsilyl)-4-(hydroxymethyl)-2-azetidinone[1b] *via*: a) Benzyloxymethyl chloride, EtN(*i*Pr)$_2$, CH_2Cl_2, r.t., 74%; b) *n*Bu$_4$NF, THF, -40°C, 99%; c) KOH, *n*Bu$_4$NBr, $BrCH_2CO_2Et$, THF, 0°C, 84%; d) $Ca(BH_4)_2$, THF-EtOH, -20°C, 91%. **2b**: From cinnamaldehyde: a) 2-aminoethanol, CH_2Cl_2, 4Å mol. sieves, 95%; b) *t*BuMe$_2$SiCl, Et_3N, , CH_2Cl_2, r.t., 92%;[12] c) $PhOCH_2COCl$, Et_3N, CH_2Cl_2, -78°C° → r.t., 50%; d) O_3, MeOH-CH_2Cl_2, -78°C, then Me_2S, then $NaBH_4$, 85%; e) BOM-Cl, *n*Bu$_4$NI, EtN(*i*Pr)$_2$, DMF, 60°C, 74%; f) HF, H_2O-CH_3CN, -15°C, 96%. **2c**: From (d,l) isopropylidene glyceraldehyde: a) *p*-anisidine, CH_2Cl_2, 4 Å mol. sieves; b) allyloxyacetyl chloride, Et_3N, CH_2Cl_2, -20°C→ r.t., 51% (only one diastereoisomer formed).[13] c) O_3, MeOH-CH_2Cl_2, -78°C, then Me_2S; d) BH_3•THF, 90%. **2d**: From (d,l) isopropylidene glyceraldehyde: a) and b) as for **2c**. c) Ceric ammonium nitrate, CH_3CN, H_2O, 60%. d) *t*BuOK, $BrCH_2CO_2$*t*Bu, *n*Bu$_4$NI, THF, -20°C, 64%. e) O_3, MeOH-CH_2Cl_2, -78°C, then Me_2S; d) BH_3•THF, 84%. **2e**: From allyl benzyloxymethyl ether: a) O_3, MeOH-CH_2Cl_2, -78°C, then Me_2S, r.t. b) *p*-anisidine, CH_2Cl_2, 4Å mol. sieves. c) allyloxyacetyl chloride, Et_3N, CH_2Cl_2, -20°C → r.t., 51%; d) O_3, MeOH-CH_2Cl_2, -78°C, then Me_2S; e) BH_3•THF, 77%. **2f**: From methyl α-(*p*-anisidinylimino)acetate: a) (*E*) crotonyl chloride, Et_3N, CH_2Cl_2, reflux.[14] b) $Ca(BH_4)_2$, THF-EtOH, 76%. c) BOM-Cl, EtN(*i*Pr)$_2$, *n*Bu$_4$NI, DMF, 50°C, 85%. d) 9-BBN, THF, r.t., then $KHCO_3$, H_2O_2, 72%.
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