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Facile N-1 protection of cyclam, cyclen and 1,4,7-triazacyclononane

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Abstract—An exceptionally high yielding method for the tri-protection of cyclam and cyclen using ethyl trifluoroacetate is described. The selective reaction also applies to the di-protection of 1,4,7-triazacyclononane. The application of this method in the synthesis of AMD3100, a clinical candidate for stem cell mobilization is presented.

The advent of the Richman–Atkins procedure,[1] and later improvements,[2] for the synthesis of polyazamacrocycles have given ready access to a wide variety of potentially useful compounds. Among the most known ones are cyclam, cyclen and 1,4,7-triazacyclonane (TACN). These macrocycles and especially their N-1 protected (N being the total identical ring amino function) derivatives have found use as starting materials in the synthesis of transition metal and lanthanide-specific chelating ligands,[3,4] MRI contrast agents,[5] radio-diagnostic and therapeutic agents.[6,7] However, selective protection of the otherwise undifferentiated amine moieties of a polyazamacrocycle can be difficult at best. As part of our effort in our drug development program involving cyclam derivatives, we have found ethyl trifluoroacetate (EtOTFA) to be an ideal reagent for the selective tri-protection of 1,4,8,11-tetraazacyclo-tetradecane (cyclam), as well as 1,4,7,10-tetraazacyclo-dodecane (cyclen), and similar di-protection of 1,4,7-triazacyclononane (TACN) under non-stoichiometrical controlled conditions.

Currently,[8,9] there are several general methods practiced to obtain differentiated, or selectively protected, cyclam derivatives. The first involves the direct reaction of a chosen protecting agent with the macrocycle, which is typically in significant excess, under optimized conditions including stoichiometry,[10] pH,[11] temperature and concentration. A compromised yield and intense purification procedure are usual outcome of this strategy owing to the inevitable formation of so-called under- and over-protected side-products.[12]

Another strategy adopts the temporary blocking of three of the four amino groups with certain transition metal-tricarbonyl,[13] boron[14] and phosphorus[15] containing moieties, leaving the free amine residue open for further manipulation. Each of these procedures has drawbacks, ranging from the use of air sensitive materials to the use of undesirable solvents.

A third approach involves differentiation prior to the macrocyclic ring-forming step.[16,17a]

During the course of our drug development program for AMD 3100 (Fig. 1), a candidate for stem cell mobilization,[18] a scalable manufacturing process for tri-*N*-protected cyclam is needed. Earlier efforts mimicked the original medicinal chemistry route using tri-tosyl cyclam as the intermediate.[12] On kilogram scale, as in the research laboratory, this tri-tosyl intermediate was obtained with a relatively low yield (<40%) and modest purity (90–95% by HPLC) after repeat recrystallization. AMD 3100 was produced in less than 10% yield from the starting cyclam (followed by *N*-alkylation with α,α′-dibromo-*p*-xylene and subsequent

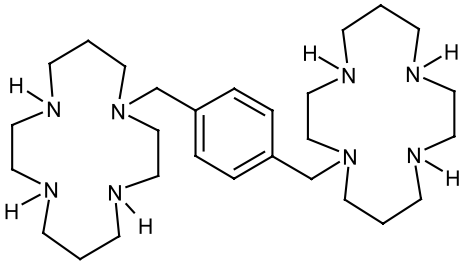

Figure 1. Structure of AMD 3100.

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removal of tosyl groups using HBr/HOAc). The long processing/reaction time, associated with the high price of cyclam, made the overall cost of final drug substance prohibitively high.

Ethyl trifluoroacetate has been frequently used as a mild acetylating agent for amine protection.[19,20] More recently, Prasad et al,[17b] further illustrated reaction examples displaying selectivity with EtOTFA among primary and secondary amines and between certain identical secondary amino groups. Interestingly, the same authors also showed a tri-protection of a linear tetraamine molecule using 3 equiv. of EtOTFA during their approach to AMD 3100[17a,21] (previously JM 3100). In addition, they reported two examples where mono-protection was achieved with good yield between two identical secondary amino groups in the same molecule. For example, when one equivalent of piperazine was treated with one equivalent of EtOTFA in THF at room temperature, the mono:di ratio in the product mixture was reported as close to 6:1. However, these selectivity observed in linear polyamines and small ring multiple amino containing compounds diminished once excess EtOTFA was used. And generally, when equal or more equivalents EtOTFA (versus free amino units present) are used, a global protection occurs.

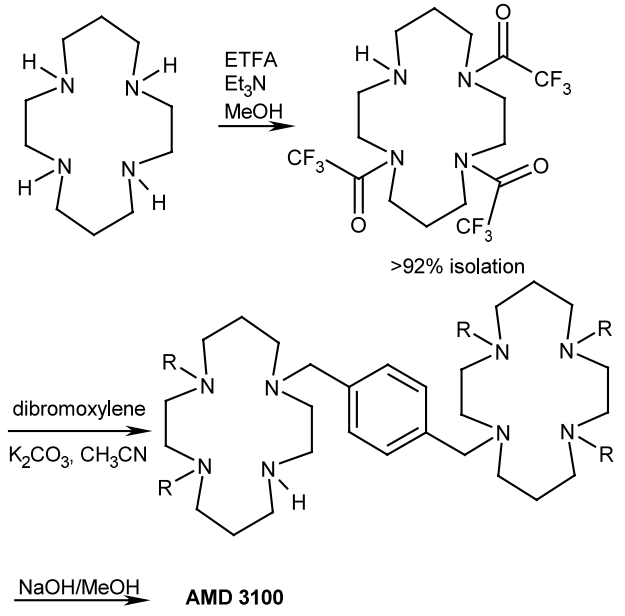
While investigating alternative cyclam protection schemes, we observed an unprecedented reaction pattern of cyclam, a so-called polyamine macrocycle, with EtOTFA. Herein, we wish to briefly disclose our findings.[22]

As expected, when 1 equiv. of cyclam was let to react with 1 equiv. of EtOTFA ($CHCl_3$, room temperature, 15 h), LC/MS analysis of the reaction mixture revealed the presence of 20% mono-, 30% mixed di- and 2% tri-protected cyclam, in addition to a small amount of unreacted cyclam itself. We were a bit surprised but not really concerned by the absence of any detectable tetra-protected cyclam. However, a separate reaction using four equivalents of EtOTFA per equivalent cyclam (CH_2Cl_2/MeOH, at 45°C, 15 h) produced a mixture of 1% mono-, 60% di- and 32% tri-substituted cyclam derivatives and no detectable tetra-TFA cyclam. This stands in sharp contrast to reactions between cyclam and other common protecting agents, such as tosyl chloride, where there is a great tendency for the formation of tetra-substituted cyclam.

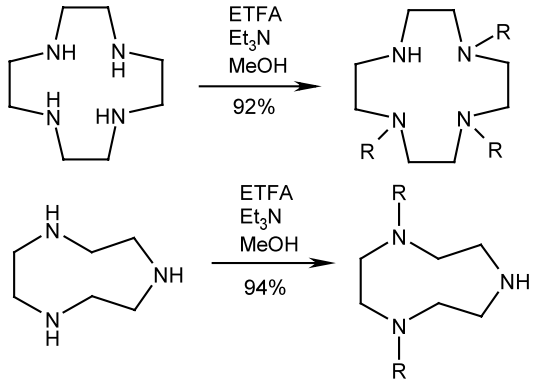
Further experiments demonstrated that only a neglectable amount of tetra-derivative was formed even when a large excess (i.e. up to using EtOTFA as the reaction solvent) of EtOTFA was used. We also found that the rate of the reaction was accelerated when MeOH was adopted as the sole solvent. In the absence of any additives, the reaction afforded 90–95% (HPLC) tri-TFA cyclam within 4 h using 10 equiv. of EtOTFA. The addition of triethylamine was found to allow the reaction to approach closer to completion, giving >99% tri-TFA cyclam under otherwise same reaction conditions[23] (Scheme 1). The addition of triethylamine, as previously used by other reports, may scavenge trifluoroacetic acid, present in EtOTFA due to hydrolysis by adventitious water, which would otherwise protonate the amino groups in the mixture. The enhancement of reaction rate by using a combination of MeOH and triethylamine is most likely resulted from polarity and base catalytic effects. Use of EtOH as solvent produced similar results, which ruled out possible base-catalyzed transesterification prior to acylation.

Adopting the new method, we have achieved the synthesis of AMD 3100 with an overall yield of >72%. Labor cost and time are both saved greatly by means of easy protection/deprotection sequence. This resulted in a much-reduced cost of the final drug substance. The use of excess low cost EtOTFA offers a better kinetic control of the reaction (accelerated rate) without posing a problem during isolation, as the excess reagent is easily distilled off. Tri-TFA cyclam has a much higher R_f value (versus various di-TFA cyclam by-products) on column chromatography. This offers an easy isolation by passing through a silica gel plug. On scale, the reaction mixture is carried on to the next step without purification. No effort was spent to recrystallize the white foam like Tri-TFA cyclam. Subsequent alkylation went uneventful in acetonitrile with K_2CO_3 as the base. Finally, straightforward mild deprotection (MeOH/aq. NaOH, rt) of the amides afforded AMD3100 free base in several hours.

Similarly, we found this protection chemistry to be effective for the selective protection of both cyclen and 1,4,7-triazacyclononane (Scheme 2). In both cases, over 90% isolation was achieved for tri-TFA cyclen and di-TFA 1,4,7-triazacyclononane, respectively. These intermediates may find applications in the preparation of currently marketed MRI agents.



Scheme 1. Improved synthesis of AMD 3100.



Scheme 2. Preparation of tri-TFA cyclen and di-TFA TACN.

In summary, we have discovered a simple, but extremely effective and economical method for the preparation of tri-protected cyclam/cyclen and di-protected TACN. The efficiency is exemplified in the improved synthesis of AMD 3100, a stem cell mobilizing agent currently in clinical trial.

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23. Procedure for the preparation of Tri-TFA cyclam: ethyl trifluoroacetate (18.0 mL, 150.3 mmol) was added dropwise to a mixture of cyclam (7.53 g, 37.58 mmol) and Et_3N (5.20 mL, 37.58 mmol) in methanol (30 mL) at room temperature. The addition continued over a period of 5 min. The homogeneous reaction mixture was cooled with an ice-water bath to control the mild exotherm. Stirring was continued under N_2 for 5 h. Volatiles were removed in vacuo. The residue was passed through a small silica gel plug (25 g), eluted with 100% EtOAc. The eluent was concentrated to give the product as a white foam (17.05 g, 92.5%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.85–3.25 (m, 12H), 2.90–2.80 (m, 2H), 2.74–2.50 (m, 2H), 2.30–1.90 (m, 2H), 1.85–1.63 (m, 2H), 1.25–0.60 (m, 1H). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 158.7–157.3 (m, C=O, existence of conformers and long range C–F coupling), 122.1 (q, CF_3, $J_{C-F} \sim 264$ Hz, further split due to conformers), 51.2–46.2 (m, CH_2-N), 29.4–27.8 (m, CH_2). ^{19}F NMR (282.4 MHz, $CDCl_3$, DMF^{d-7}, 120°C): 7.50 (s), 6.88 (s), 6.60 (s). Anal. calcd for $C_{16}H_{21}F_9N_4O_3$: C, 39.35; H, 4.33; N, 11.47; found: C, 39.19; H, 4.36; N, 11.33. MS calcd (M+1) 489.2, found 489.1. The proton-decoupled ^{13}C NMR is complicated by C–F coupling (including long range) and the existence of conformers at room temperature.