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# Highly regioselective N-trans symmetrical diprotection of cyclen

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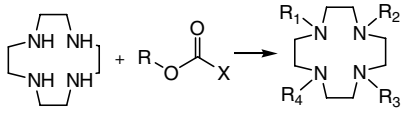
**Abstract**—A high yielding N-trans diprotection procedure for cyclen has been developed by using succinimide carbamate derivates of Boc and Cbz and chloroformate protecting groups.


Polyaza macrocyclic systems are widely studied due to their metal complexation properties. Particularly, the N-functionalization of tetraaza macrocyclics such as 1,4,7,10-tetraazacyclododecane (cyclen) has been widely pursued since the generated derivatives have wide application as contrast agents in MRI,[1] radiodiagnostic and radiotherapeutic agents,[2] fluorescent sensors[3], etc. In general, N-derivatization has been accomplished by following two approaches: direct derivatization and protection–derivatization–deprotection. Literature reports on mono-, bis- and tri-derivatization by either approach are known. However, bis-derivatization procedures are scarce and are of particular interest given the possibility of obtaining either symmetrical or unsymmetrical N-trans and N-cis polyaza macrocyclic derivatives. Thus, bis-alkylated symmetrical and asymmetrical N1,N7 (N-trans) cyclen compounds have been prepared via phosphoryl[4] and silicon[5] cyclen intermediates. Additionally, N-trans symmetrically alkylated cyclen derivatives have been reported when reacting cyclen Co or Cr complexes with iodoalkanes.[6] N1,N7 symmetrical diprotection of cyclen has also been reported by following a close pH control between 2 and 3 during slow addition of chloroformates.[7] The synthesis of N-trans symmetrical and asymmetrical cyclen derivatives has also been reported by reacting alkyl halides with the intermediate perhydro-3,6,9,12-tetraazacyclopenteno[1,3-f,g]acenaphthylene in a suitable solvent.[8] On the other hand, N1,N4 (N-cis) cyclen derivatives have been synthesized following protection[9] and direct derivatization approaches.[10] However, the protection–functionalization–deprotection based methods have two main disadvantages: first, the low to moderate overall yields (from 48% to 78%) for the final functionalized cyclen derivative and second, the harsh conditions required for the removal of some of the suggested protecting groups. Therefore, direct functionalization methods are often preferred. While these work reasonably well for mono and trisubstitution, direct functionalization of cyclen to obtain disubstituted derivatives usually shows lack of regioselectivity and a mixture of mono-, di-, tri- and tetra-functionalized products is obtained, making their purification difficult.

Given the need for an efficient methodology to prepare di-functionalized cyclen derivatives in this work, we studied the regioselectivity of several reagents commonly used in peptide synthesis to produce readily cleavable *tert*-butoxycarbonyl and benzyloxycarbonyl protected derivatives (Scheme 1). We have found that




$R_1=R_2=R_3=$ -H, $R_4=$ $-CO_2R$ N-mono
$R_1=R_3=$ -H, $R_2=R_4=$ $-CO_2R$ N1,N7-di
$R_1=$ H, $R_2=R_3=R_4=$ $-CO_2R$ N-tri
$R_1=R_2=R_3=R_4=$ $-CO_2R$ N-tetra

**Scheme 1.** Regioselective N-protection of cyclen. Where R = benzyl or *tert*-butyl and X = chloride, –OCOBn or oxysuccinimide.

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N1,N7 diprotected products can be obtained in nearly quantitative yields when the corresponding (oxycarbonyloxy)succinimide reagents are used.

To understand the origin of the regioselectivity of these N protection reactions, it is necessary to know the structure-reactivity characteristics of this unique macrocyclic system. However, there are few literature reports on this aspect although the protonation constants ($pK_a$ values) of the four amino groups are known to be 10.5, 9.5, 1.6 and 0.8, respectively.[11] These values give a relative measure of the basicity of the amino groups. Moreover, it is important to consider that they indicate consecutive protonation steps, which in turn causes conformational changes to keep apart the positively charged amino groups. The latter has been observed in the X-ray structures of the free base[12] and the tetra-protonated cyclen.[13] Thus, based on the knowledge of $pK_a$ values, it has been shown that cyclen can be N1,N7 diprotected with benzyloxycarbonyl (Cbz) in aqueous media with good yields (88%) by keeping the pH between 2 and 3 during slow addition of chloroformates.[7] While this protection gives reasonable yields, the slow addition of the chloroformate reagent is inconvenient, especially when the protection is carried out on a large-scale basis. In addition, this procedure is limited to non-acid labile protecting groups as the reaction is run in fairly acid solutions. In an attempt to develop a more practical method of cyclen protection, we assumed that the cylic tetramine adopts a similar structure in solution as the cyclen free base in the solid state. This has been shown to be a [3333] quadrangular conformation with C atoms occupying corner positions by X-ray structure determination. Additionally, the H atoms bound to N1,N7 are directed inward toward the center of the ring while H atoms bound to N4,N10 are pointed outward, away from the center of the ring. Considering this, cyclen has two amino groups with freely available pair of electrons and therefore the addition of 2 equiv of chloroformate reagent should give a N1,N7 diprotected product. Furthermore, if one or both of the non protected amino groups N4,N10 are basic enough then the diprotected cyclen hydrochloride should be formed and precipitated from the reaction mixture in a non-polar solvent. To test this idea, we reacted 1 equiv of cyclen with 2 equiv of benzyl chloroformate (Cbz-Cl) in chloroform. The reaction proceeded quickly with precipitate formation. Thus after reaction completion, the diCbz-cyclen dihydrochloride was obtained nearly quantitatively. This result confirms that the diprotected cyclen product is acting as a base in the reaction, followed by its precipitation due to its low solubility in chloroform which also prevents further reaction. To study the effect of the solvent, the reaction was carried in acetonitrile yielding 73% N1,N7-di protected cyclen with the rest being tri- and tetra-protected products. The decrease in the yield can be attributed to the slightly higher solubility of the hydrochloride product in acetonitrile. This observation provides a very simple procedure for the synthesis of 1,7-bis(benzyloxycarbonyl)-1,4,7,10-tetraazacyclodo-

**Table 1.** Yields for the reaction between cyclen and dicarbonates in chloroform at room temperature

| R | Cyclen: R-X | N-mono | N1,N7-di | N-tri | N-tetra |
|---|---|---|---|---|---|
| Cbz | 1:2 | — | 35.4 | 59.6 | 5.0 |
| | 1:3 | — | 42.1 | 44.4 | 13.5 |
| Boc | 1:2 | — | — | 92.4 | 7.6 |
| | 1:3 | — | — | 95.3 | 4.7 |

X = –OR.

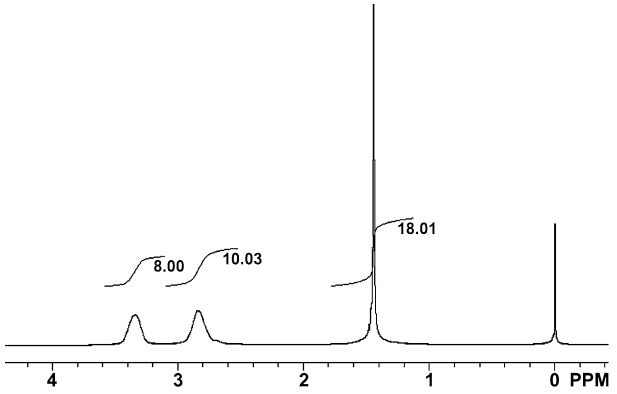



**Figure 1.** $^1$H NMR spectra of N1,N7 diBoc-cyclen.

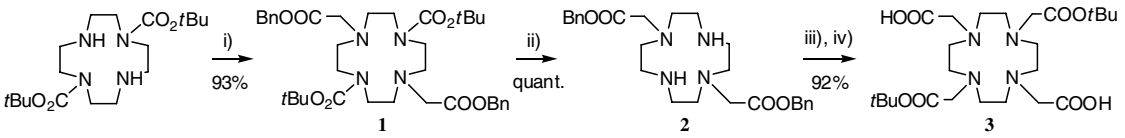
**Table 2.** Yields for the reaction between cyclen and succinimide protecting groups at room temperature (unless otherwise indicated) in different solvents

| Solvent | R Cyclen: R-X | Cbz | | | | Boc | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | N-mono | N1,N7-di | N-tri | N-tetra | N-mono | N1,N7-di | N-tri | N-tetra |
| $CH_3Cl$ | 1:2 | — | 97.6 | 2.4 | — | — | 99.5 | 0.5 | — |
| | 1:2[a] | — | 94.4 | 1 | 4.6 | — | 99.4 | 0.6 | — |
| | 1:3 | — | 94.8 | 2.1 | 3.1 | — | 100 | — | — |
| | 1:2[b] | | 91.8 | 3.5 | 4.7 | — | 99.9 | 0.1 | — |
| $CH_3CN$ | 1:2 | — | 93.9 | 4.8 | 1.3 | — | 98.9 | 1.1 | — |
| | 1:2[a] | — | 90.9 | 6.5 | 2.6 | — | 98.9 | 1.1 | — |
| | 1:3 | — | 91.2 | 7.0 | 1.8 | — | 100 | — | — |
| MeOH | 1:2 | — | 95.9 | 4.1 | — | — | 100 | — | — |
| | 1:2[a] | — | 96.7 | 3.3 | — | — | 99.7 | 0.3 | — |
| | 1:3 | — | 91.7 | 6.7 | 1.6 | — | 100 | — | — |

X = –OSucc.

[a] Plus 2 equiv of DIPEA.

[b] Temperature 60 °C.




**Scheme 2.** Reagents and conditions: (i) $BrCH_2COOBn$, $K_2CO_3$, $CH_3CN$, (ii) $CF_3COOH$, $CH_2Cl_2$, (iii) $BrCH_2COOtBu$, $K_2CO_3$, $CH_3CN$, (iv) $H_2$, Pd/C, EtOH.

decane. However, the introduction of other protecting groups such as Boc might be limited due to the lack of the proper reagent.

Thus continuing with our quest, we tested dibenzyl and di*tert*-butyl dicarbonate for the selective protection of cyclen using chloroform as a solvent and the results are summarized in Table 1.

The reaction of cyclen with di*tert*-butyldicarbonate gives the N-tri-protected product in high yield as reported previously.[14] On the other hand, reaction with dibenzyldicarbonate is poorly regioselective, yielding a mixture of N1,N7-di- and N-tri- and N-tetra-protected products. This interesting difference in the observed regioselectivity is likely due to the intramolecular steric effect inherent in the protecting group. Thus, it is expected that the bulkier Boc induces more steric hindrance, which explains the lower amount of N-tetra protected product compared to Cbz protection. The basicity of the leaving group in the protecting reagent may also influence the regioselectivity. If the basicity of the leaving group is comparable to or higher than that of the unprotected amino groups of cyclen, then the equilibrium will shift to the formation of the conjugate acid of the leaving group. Hence, the deprotonated product can lead to the formation of tri- and tetra-protected derivatives.

Therefore, if the desired product is diBoc-cyclen then the reagent used must have a weakly basic leaving group. Oxycarbonyloxysuccinimide reagents were selected due to the relatively poor basicity of the oxysuccinimide (OSucc) leaving group (hydroxysuccinimide, $pK_a = 7.8$).[15a] Indeed, it was observed that both *tert*-butyl- and benzyl-(oxycarbonyloxy)succinimide (Boc-OSucc and Cbz-OSucc, respectively) yielded the N1,N7-di-protected cyclen product almost quantitatively (see Fig. 1). Particularly, the *tert*-butoxycarbonyl diprotection is unaffected by the addition of extra reagent, the addition of an organic base, the change of solvent nor the change in temperature. The results are summarized in Table 2.

N1,N7 diprotection with Cbz-OSucc, however, is slightly more dependent on the reaction conditions. The somewhat lower yield for the Cbz protection using Cbz-OSucc may be attributed to a decrease in steric hindrance when compared to the Boc-OSucc reagent.

In additional experiments using Cbz-benzotriazoxy and pentaflurophenoxy[16] (benzotriazol and pentafluorophenol $pK_a = 7.4$[15b] and 5.5[15c]) derivatives, the N1,N7 di-protected cyclen derivatives were also obtained quantitatively.

Unfortunately, the (oxycarbonyloxy)succinimide reagents did not show any regioselecivity with piperazine and 1,4,8,11-tetraazacyclotetradecan (cyclam). With piperazine, the diprotected product was observed in high proportion and in the case of cyclam, a mixture of compounds was obtained.

The utility of the present methodology was demonstrated with the synthesis of DOTA-bis(*tert*-butyl) ester) **3** (Scheme 2), which is a useful intermediate for the synthesis of new cyclen derivatives. To prepare **3**, the 1,7-bis(benzyloxycarbonyl)-cyclen intermediate was alkylated at the 4 and 10 positions with benzyl bromoacetate, followed by the removal of Boc groups in acidic media to yield compound **2**. Intermediate **2** is then alkylated with *tert*-butyl bromoacetate in the presence of potassium carbonate followed by the removal of the benzyl groups by catalytic hydrogenation. Thus, **3** was prepared with an overall yield of 86%.

In summary, we have discovered a simple and highly regioselective method for the preparation of the synthetically useful 1,7 bis(*tert*-butyl)[17] and bis(benzyloxycarbonyl)[18] cyclen derivatives.

## Acknowledgements

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15. (a) Calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris (1994-2006 ACD/Labs); (b) Boyle, F. T.; Jones, R. A. Y. *J. Chem. Soc., Perkin. Trans. 2* **1973**, 160; (c) Birchall, J. M.; Haszeldine, R. N. *J. Chem. Soc.* **1959**, 3653.
16. Benzylpentafluorophenylcarbonate: To a solution of pentafluorophenol (130 mg, 0.71 mmol) and diisopropylethylamine 136.1 μL (0.78 mmol) in dichloromethane (8 mL) was added benzyl chloroformate (Fluka) (100 mg, 0.8 equiv). The solution was stirred a room temperature under nitrogen atmosphere and monitored by TLC. Product was purified by column chromatography (silica gel, dichloromethane). After solvent removal the product was obtained quantitatively as a clear oil.
$^{1}$H NMR (200 MHz, $CDCl_3$/TMS): $\delta$ 7.41 (5H, s arom.), 5.32 (2H, s, $-CH_2-$). $^{13}$C{1H} NMR (200 MHz, $CDCl_3$): $\delta$ 151.4 ($-CO_2-$), 144.1, 140.4, 138.7, 135.3 (C–F), 133.8, 129.2, 128.9, 128.6 (arom.).
17. Typical procedure for the synthesis of N1,N7 diprotected cyclen derivatives from succinimide reagents: To a solution of tetraazacyclododecane free base (714 mg, 4.067 mmol) in chloroform (35 mL) was added *N*-(Benzyloxycarbonyloxy)succinimide or *N*-(*tert*-Butoxycarbonyloxy)succinimide (Fluka) (2 equiv). The reaction mixture was stirred at room temperature for 1–2 days. Solvent was removed by rotary evaporation and 30 mL of NaOH 3 M was added to the remaining residue. The aqueous phase is extracted with chloroform ($3 \times 30$ mL). The extracts were combined and dried ($K_2CO_3$). The solvent was removed by rotary evaporation and the residue was dried in vacuum for several hours.
1,7-Bis(*tert*-butyloxycarbonyl)-tetraazacyclododecane: $^{1}$H NMR (200 MHz, $CDCl_3$/TMS): $\delta$ 3.35 (8H, br m, $-CH_2-$ N), 2.83 (10H, br m, $-CH_2-$N, –N*H*–), 1.45 (18H, s, $-CH_3$). $^{13}$C{1H} NMR (200 MHz, $CDCl_3$): $\delta$ 155 ($-CO_2-$), 76.45 ($(CH_3)_3C$–O), 50.9 ($-CH_2-$N), 48.3 ($-CH_2-$N), 28.4 ($-C(CH_3)_3$. Anal. Calcd for $C_{18}H_{36}N_4O_4$: C, 58.04; H, 9.74; N, 15.04. Found: C, 58.11; H, 9.82; N, 15.12.
1,7-Bis(benzyloxyycarbonyl)-tetraazacyclododecane: $^{1}$H NMR (200 MHz, $CDCl_3$/TMS): $\delta$ 7.34 (10H, s, arom. Bn), 5.15 (4H, s, $-CH_2-$, Bn) 3.41 (8H, br m, N–$CH_2-$), 3.04 (2H, s, –NH–), 2.83 (8H, br m, N–$CH_2-$). $^{13}$C{1H} NMR (200 MHz, $CDCl_3$): $\delta$ 158 ($-CO_2$), 138 (–CH–, arom), 128 (–CH– arom), 127 (–CH–, arom.), 67 ($-CH_2-$O), 52–48 ($-CH_2-$N). Anal. Calcd for $C_{24}H_{32}N_4O_4$: C, 65.43; H, 7.32; N, 12.72. Found: C, 65.32; H, 7.35; N, 12.70.
18. Typical procedure for the synthesis of 1,7-Bis(benzyloxycarbonyl)-tetraazacyclododecane from benzylchloroformate: To a solution of tetraazacyclododecane free base (671 mg, 3.39 mmol) in chloroform (35 mL) in an ice bath was added benzyl chloroformate (Fluka) (2 equiv) dropwise. Reaction was stirred overnight giving abundant solid formation. Solvent was removed by rotary evaporation and ether (30 mL) was added. The solid was filtered, washed with ether and dried yielding 1.735 g (100%) of the dihydrochloride salt as a white solid. The free base was obtained by adding NaOH (30 mL, 3 M) to the solid The aqueous phase was extracted with chloroform ($3 \times 30$ mL). The extracts were combined and dried ($K_2CO_3$). The solvent was removed by rotary evaporation and the residue was dried under vacuum for several hours giving 1.449 g (100%) of a transparent oil. The characterization data corresponded to those indicated in Ref. 17.