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Regioselective *N*-substitution of cyclen with two different alkyl groups: synthesis of all possible isomers†

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All possible configurations of two different groups on the four nitrogen atoms of cyclen were achieved using four differently protected cyclen intermediates including the novel mono-protected cyclen compound, mono-*N*-Cbz-cyclen.

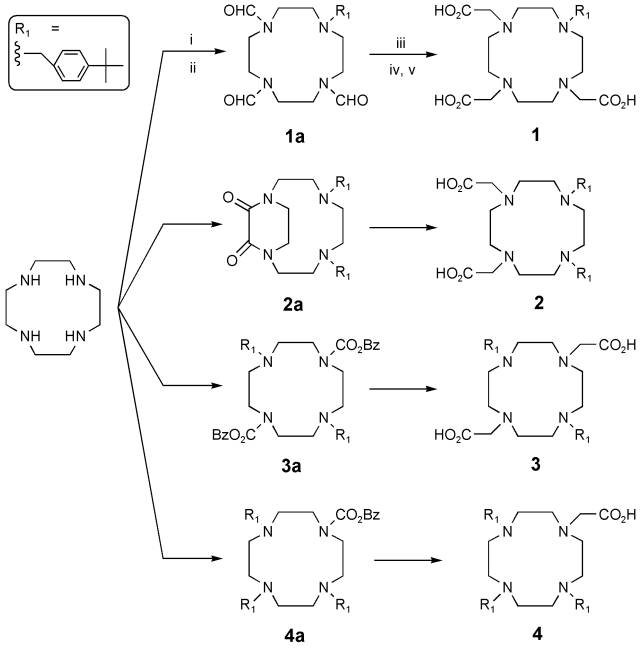
Current interest in the regioselective *N*-functionalization of 1,4,7,10-tetraazacyclododecane (cyclen) stems mainly from their complexes with radioactive metals for applications in diagnostic (^{64}Cu, ^{111}In, ^{67}Ga) and therapeutic (^{90}Y) medicine,[1] and with paramagnetic ions for magnetic resonance imaging (Gd^{3+}).[2] Most recently, tetraazamacrocycles have found applications as anti-tumor[3] and anti-HIV agents.[4] Selective methods for the *N*-substitution of cyclen is a crucial step in most syntheses of cyclen-based ligands and bifunctional chelating agents. In addition, mixing different pendent groups, especially hydrophilic and hydrophobic groups, to give hetero-substituted cyclen derivatives would be advantageous in many applications for fine-tuning the compound's physical properties.[5,6] Small changes in structure can result in huge differences in physiological properties of medicinal agents. SARs (structure activity relationships) based on selective modification of structure are well known to be a powerful strategy in new drug development. With coordination complexes of cyclen the number of acetic acid groups (CH_2CO_2H) introduced onto the nitrogen atoms has a huge effect on the coordination geometry and final formal charge under physiological conditions, affecting both the thermodynamic stability and kinetic inertness.[7] The cyclen derivatives DO3A (three acetates) and DOTA (four acetates) are the most widely used cyclen-based compounds for medicinal applications.

To obtain different substitution patterns, we utilize a strategy of regioselective protection/1st alkylation/deprotection/2nd alkylation (Scheme 1). Direct functionalization and cyclization of *N*-alkylated precursors can be applied in some cases, but a protection/functionalization/deprotection method is a more reliable and efficient way in most cases.[8] The protecting groups used are required to be introduced regioselectively among the four identical nitrogen atoms of cyclen in high yield and easily cleaved in mild reaction conditions without attacking other functional groups. Herein we report efficient synthetic methods for all possible hetero-substituted cyclen isomers which could be synthesized using two different pendent arms with at least one acetic acid group. We also report a facile and effective way to prepare mono-protected cyclen, which is a very useful synthon for preparing DO3A derivatives, and the methylene phosphonic acid ($CH_2PO(OH)_2$) analogs, DO3P derivatives.

Different numbers of cyclen's nitrogens were protected regioselectively to afford mono-, *cis*-di- and *trans*-di-, and tri-nitrogen protected cyclen compounds. These protected species were then reacted with 4-(*tert*-butyl)benzyl bromide to give the alkylated products (**1a**–**4a**). The subsequent functionalization with acetic acid groups was carried out after deprotection to give final products which have two different pendent arms (**1**–**4**).

A tri-protected cyclen compound was prepared by the reaction of cyclen and chloral hydrate without difficuties.[9] Triformylcyclen was reacted with the alkylating group, 4-(*tert*-butyl)benzyl bromide in the presence of iPr$_2$NEt in acetonitrile to give the mono-alkylated product (**1a**), which was purified by simple recrystallization in diethyl ether. Due to a restricted rotation of the N–CHO bond, the ^{1}H and ^{13}C NMR spectra of the triformylcyclen compound were more broad and complicated than expected, and the compound was characterized by elemental analysis and HR-MS. Deprotection utilizing base as in the original paper[9] was ineffective in our case. Treatment with base (1 M KOH, 80 °C, 15 h) was found to give incomplete removal of the formyl groups. Instead, deprotection of the formyl groups was found to be easily accomplished using acidic conditions (2 M HCl, 60 °C). Evaporation of the aqueous solution gave the pure product as the HCl salt. Subsequent introduction of *tert*-butyl bromoacetate was achieved using iPr$_2$NEt as base in acetonitrile similar to the first alkylation step. Deprotection of the *tert*-butyl groups using hydrochloric acid (6 M) afforded the tri-acetate DO3A derivative (**1**). By choosing different initial alkylating groups, many useful DO3A derivatives for BFCs (bifunctional chelates) might be produced.[10]

Two disubstituted cyclen isomers, 1,4- and 1,7-dialkylated cyclen derivatives, were prepared successfully in high yield using diethyl oxalate and benzyl chloroformate as protecting groups following literature methods, which led to the formation of cyclenoxamide[11] and 1,7-dibenzyloxycarbonyl-cyclen,[12] respectively. Both *cis*- and *trans*-protected intermediates (with respective to the relative positions of tertiary nitrogen atoms)



Scheme 1 i, Protection; ii, 1st alkylation ((4-(*tert*-butyl)benzyl bromide, iPr$_2$NEt, MeCN); iii, deprotection; iv, 2nd alkylation ($BrCH_2CO_2tBu$, iPr$_2$NEt, MeCN); v, deprotection.

† Electronic supplementary information (ESI) available: spectroscopic data. See http://www.rsc.org/suppdata/cc/b2/b212667b/

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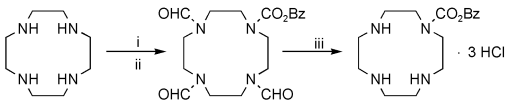
were alkylated in the same way as above (**2a** and **3a**). Subsequent deprotection and the second alkylation followed to afford *cis*- and *trans*-DO2A derivatives (**2**, **3**) in 25–26% overall yield based on starting cyclen. The reaction products were purified by either recrystallization (**2a**, **2** and **3**) or column chromatography (alumina, ethyl acetate for **3a**).

To prepare trialkylated cyclen derivatives, we needed to devise an effective way to differentiate one of the nitrogen atoms of cyclen. Although there are a few reports describing mono-*N* protection, these methods use an excess of relatively expensive cyclen, followed by laborious chromatographic separation of mono-protected cyclen from di- and tri-protected cyclen compound mixtures which have very similar R_f values,[6] or protect three nitrogen atoms first using *N*,*N*-dimethylformamide dimethyl acetal,[13] Ts[13] or Boc[5] followed by conversion of these intermediates to mono-protected cyclen compounds. In these methods, the overall yield of mono-protection based on cyclen is in the range of 25–50% owing to the low yield of the protection step and the requisite chromatographic purification of cyclen compounds. Our method utilizes a one-pot reaction by first protecting three nitrogens with identical protecting groups followed by protection of the fourth nitrogen. The three identical protecting groups are subsequently removed to give mono-protected cyclen with an easily removable benzyl carbamate group (mono-*N*-Cbz-cyclen) (Scheme 2).



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Scheme 2 Reagents and conditions: i, chloral hydrate, EtOH, 60 °C; ii, benzyl chloroformate, H_2O; iii, 1 M HCl, 50 °C.

Three of the four nitrogen atoms of cyclen were protected by formyl groups by reaction with four equivalents of chloral hydrate in ethanol.[9] We have found that four equivalents of chloral hydrate relative to cyclen is enough to protect only three nitrogen atoms of cyclen selectively but does not produce other possible isomers such as mono-, di- and tetra-protected cyclen compound. After evaporation of solvent to dryness, the product was further reacted with an excess of benzyl chloroformate without any purification. The pH of the aqueous mixture was continuously maintained in the range of pH 4–9 for complete reaction. The crude product was extracted with methylene chloride and simply recrystallized from diethyl ether to give pure 1,4,7-triformyl-10-(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane‡ (**5**) in quantitative yield. Due to the hindered rotation of three formyl groups, the peaks in the ^{1}H NMR spectrum are broad, and more resonances than expected are observed in the ^{13}C NMR spectrum.[9] The compound, **5**, was also ascertained by TLC [R_f = 0.39 on alumina, MeOH : CH_2Cl_2 (1 : 20)], elemental analysis and HR-MS. Mild acidic treatment (1 M HCl, 50 °C) of **5** led to selective cleavage of three formyl groups without attacking the Cbz group. Elevated temperature and higher acidity could break the Cbz–N bond to return to the starting cyclen itself. After complete evaporation of the aqueous solution the crude product was recrystallized from EtOH/Et_2O to give the pure mono-protected cyclen compound, 1-(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane as the hydrochloride salt§ (**6**). The overall yield for the two reactions based on cyclen is over 80%, which is much higher than the previous mono-protection methods. The benzyloxycarbonyl group itself has advantages as a protecting group for nitrogen atoms because it can be easily removed by catalytic hydrogenation in addition to normal acidic hydrolysis.

The mono-protected compound 1-(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane was then alkylated using the previously described method to give a mono-protected and tri-alkylated cyclen compound (**4a**). Removal of the benzyloxycarbonyl protecting group by hydrogenation over Pd/C afforded the tri-alkylated cyclen compound. The second alkylation with *tert*-butyl bromoacetate, followed by deprotection of the *tert*-butyl group, led to theDO1A derivative (**4**). This mono-protected cyclen also provides easy access to important intermediates, DO3A–$(OR)_3$ (R = *t*Bu, Et, Bz) for the synthesis of DOTA- and DO3A-based BFCs.[14]

In conclusion, all possible isomers with two different pendents, DO1A, *cis*- and *trans*-DO2A and DO3A derivatives, were synthesized by employing the same synthetic strategies. A judicious choice of four different protecting groups was found to be essential to the high-yield preparation of regioselective *N*-alkylated cyclen compounds. All compounds are fully characterized by ^{1}H NMR, ^{13}C NMR, and either elemental analysis or high resolution mass spectroscopy. A mono-*N*-protected cyclen compound, mono-*N*-Cbz-cyclen, was successfully prepared in high yield without tedious chromatographic separation by one-pot consecutive reactions of cyclen with chloral hydrate and benzyl chloroformate and selective deprotection of the formyl groups. All protection and deprotection steps are air-stable and do not need harsh reaction conditions. Mono-*N*-Cbz-cyclen could be used as a synthon in the preparation of a variety of BFCs.

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Notes and references

‡ ^{1}H NMR ($CDCl_3$) δ 3.02–3.65 (m, 16H), 5.07 (s, 2H), 7.27 (br s, 5H), 7.88–8.09 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 43.0, 43.7, 43.8, 44.1, 44.7, 45.0, 45.8, 46.7, 46.9, 47.6, 48.1, 48.8, 49.4, 49.8, 50.1, 50.4, 50.5, 50.8, 52.2, 53.5 (cyclen ring *C*H_2), 67.5, 67.6 (*C*H_2Ph), 127.9, 128.3, 128.6, 129.0 (Ph ring *C*H), 135.8 (Ph ring *C*), 155.9, 156.8, 157.5 (*C*$O_2$$CH_2$Ph), 163.0, 163.4, 163.6, 163.8, 163.9, 164.3, 164.6, 165.1, 165.7, 165.9 (NCHO). Anal. Calcd for $C_{19}H_{26}N_4O_5 \cdot H_2O$: C, 55.87; H, 6.91; N, 13.72. Found: C, 55.31; H, 6.68; N, 13.04%. HRMS (ESI): *m*/*z* 413.1791 ([M + Na]$^+$, $C_{19}H_{26}N_4O_5Na$, calcd 413.1801).

§ ^{1}H NMR (D_2O) δ 3.19 (br s, 12H), 3.69 (br t, *J* = 5.1 Hz, 4H), 5.17 (s, 2H), 7.44 (br s, 5H); ^{13}C NMR (D_2O) δ 45.8, 47.5, 48.2, 49.5, 71.6 (*C*H_2Ph), 131.4, 131.8, 131.9, 138.7, 161.4 (*C*$O_2$$CH_2$). Anal. Calcd for $C_{16}H_{26}N_4O_2 \cdot 3HCl$: C, 46.22; H, 7.03; N, 13.47; Cl, 25.58. Found: C, 45.67; H, 7.42; N, 12.94; Cl, 25.34%. HRMS (FAB): *m*/*z* 307.2139 ([M + H]$^+$, $C_{16}H_{27}N_4O_2$, calcd 307.2134).

1 C. J. Anderson and M. J. Welch, *Chem. Rev.*, 1999, **99**, 2219.
2 P. Caravan, J. J. Ellison, T. J. McMurry and R. B. Lauffer, *Chem. Rev.*, 1999, **99**, 2293.
3 J. W. Sibert, A. H. Cory and J. G. Cory, *Chem. Commun.*, 2002, 154.
4 X. Liang, J. A. Parkinson, M. Weishaeupl, R. O. Gould, S. J. Paisey, H.-s. Park, T. M. Hunter, C. A. Blindauer, S. Parsons and P. J. Sadler, *J. Am. Chem. Soc.*, 2002, **124**, 9105.
5 S. Aoki, H. Kawatani, T. Goto, E. Kimura and M. Shiro, *J. Am. Chem. Soc.*, 2001, **123**, 1123.
6 L. L. Chappell, D. A. Voss Jr., W. D. Horrocks Jr. and J. R. Morrow, *Inorg. Chem.*, 1998, **37**, 3989.
7 A. Bianchi, L. Calabi, C. Giorgi, P. Losi, P. Mariani, D. Palano, P. Paoli, P. Rossi and B. Valtancoli, *J. Chem. Soc., Dalton Trans.*, 2001, 917.
8 F. Denat, S. Brandes and R. Guilard, *Synlett*, 2000, 561.
9 V. Boldrini, G. B. Giovenzana, R. Pagliarin, G. Palmisano and M. Sisti, *Tetrahedron Lett.*, 2000, **41**, 6527.
10 S. Liu and D. S. Edwards, *Bioconjugate Chem.*, 2001, **12**, 7.
11 F. Bellouard, F. Chuburu, N. Kervarec, L. Toupet, S. Triki, Y. Le Mest and H. Handel, *J. Chem. Soc., Perkin Trans. 1*, 1999, 3499.
12 Z. Kovacs and A. D. Sherry, *J. Chem. Soc., Chem. Commun.*, 1995, 185.
13 D. D. Dischino, E. J. Delaney, J. E. Emswiler, G. T. Gaughan, J. S. Prasad, S. K. Srivastava and M. F. Tweedle, *Inorg. Chem.*, 1991, **30**, 1265.
14 J. Yoo, D. Reichert and M. J. Welch, unpublished results.