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Preparation of Azide Biosynthetic Surrogates of myo-Inositol

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As a prelude to biomolecular incorporation studies, practical routes to a series of four regiosomeric azido-deoxy derivatives of inositol that mimics the natural myo-stereochrometry are described. Starting from commercially available myo-inositol, the regioselective and stereoselective introduction of azide functionality was achieved at the C-2, C-3, C-4 and C-5 positions via azide displacement of the corresponding O-sulfonates of suitably protected scyllo-chiro-, epi-, and neo-inositoles, respectively. Notably, a final one-pot acetylation method conveniently allowed for rapid access to pentaacetate azido-deoxy inositols. Investigations on the metabolic incorporation of these myo-inositol azide surrogates in both acetate and free alcohol forms are in progress.

Figure 1. Azido-deoxy surrogates (2-5) of myo-inositol (1).

A few routes to the synthesis of optically active azido and amino inositols have been reported. Examples of routes start from p-benzoquinone\textsuperscript{6} or conduritol-B\textsubscript{E}\textsubscript{2} via chemo-enzymatic resolution, and also from chiral sources such as L-quebrachitol.\textsuperscript{7} Among various synthetic protocols developed for the synthesis of myo-inositol intermediates and its analogues, commercially available myo-inositol 1 is the most commonly preferred starting material due to its low cost and pre-defined relative stereochemistry.\textsuperscript{8} For myo-inositol, positions C\textsubscript{1}, C\textsubscript{3} and C\textsubscript{5} are equivalent and unsymmetrical protection leads to racemates. The chemical synthesis of optically active inositol analogues would thus necessitate the resolution of racemic inositol intermediates (chemically, enzymatically or via desymmetrization techniques)\textsuperscript{9} or by starting with an alternative chiral material (e.g., via the Ferrier carbo-cyclization of sugars).\textsuperscript{10} For our biological studies, the racemic azido-inositol series 3 and 4, and the meso series 2 and 5, were considered sufficient to test our hypothesis of metabolic selection and incorporation into live cells. Our synthetic routes to make 2-5 are described herein.

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Synthesis of (±)-4-Deoxy-4-Azido myo-Inositol 4:

We first decided to pursue the dicyclohexylidene diketal protection approach reported by Angyal and co-workers\(^\text{12,13}\) as a starting material over diacetals or disopropylidene derivatives.\(^\text{12,13}\) This allows for the practical, differential hydroxyl protection of myo-inositol (1) as relatively stable trans and cis cyclohexylidenes that not only tolerate multi-step synthesis, but also result in reactivity differences between the two remaining hydroxyl groups. The 1,2,4,5-dicyclohexylidene myo-inositol 6 was isolated as a white solid in 22% yield after recrystallisation from 1/9 acetone/petroleum ether.\(^\text{14}\) The remaining two isomeric ketals (2,3,4,5- and 3,4,5,6-dicyclohexylidenes) can be converted to 6 in a sequence of partial deprotection-reprotection steps.\(^\text{15}\) Although yields are low, this method is scalable to multi-grams and is convenient in practice.

![Scheme 1. Synthesis of (±)-4-azido myo-inositol analogues 4a,b. Reagents and conditions: i. Cyclohexanone, toluene/DMF (1:2), pTSA, 110 °C, 22%; ii. BuO, Ba(OH)\(_2\)·2H\(_2\)O, BuBr, DMF, 60%; iii. DMP, DCM, rt, 93%; iv. NaBH\(_4\), EtOH, 0 °C, 76%; v. Tf\(_2\)O, Py, DCM 0 °C to rt, 60%; vii. TBAF, THF, rt, 99%.][Scheme1]

![Scheme 2. Synthesis of meso-5-Deoxy-5-Azido myo-Inositol 5: Reagents and conditions: i. NaH, DMF, BuBr 0 °C, 97%; ii. CH\(_2\)C\(_3\), DCM, MeOH (3:1), 68%; iii. BuBr, BuNHSO\(_4\), DCM, 5% NaOH, reflux; iv. NaH, DMF, Me, 0 °C to rt, 90%; v. DCM/MeOH (1:1), CH\(_2\)C\(_3\), rt, 89%; vi. (i), vii. DMP, NaHCO\(_3\), DCM, rt, 85%; viii. NaBH\(_4\), EtOH, rt, 60%; ix. Tf\(_2\)O, Py, 0 °C, 71%; x. Na\(_2\)CO\(_3\), DMF, rt, 83%; xi. Ac\(_2\)O, AcOH, 10% H\(_2\)SO\(_4\) in Ac\(_2\)O, rt; xii. NaOMe, MeOH, rt, 98%.][Scheme2]

Barium oxide and barium hydroxide chelation-mediated benzylisation of 6 produced the monobenzylated myo-inositol derivative 7 as the major product in 60% yield (Scheme 1). The free alcohol at the C\(_6\) position of the myo-inositol derivative 7 was subsequently oxidised under Dess–Martin periodinane (DMP) conditions to give the desired ketone 8 in excellent yield (93%). Stereoselective reduction of 8 by sodium borohydride produced the epi-alcohol 9 exclusively. The free C\(_6\) alcohol of epi-inositol 9 was subsequently activated as its triflate 10 using trifluoromethane sulfonic acid anhydride in pyridine.\(^\text{16}\) In accordance with the report of Schlewer et al.,\(^\text{17}\) azide deplacement of triflate 10 gave the desired myo-product 11 together with trace amounts of the enol ether side-product 12 via 1,2-ene elimination under the basic conditions.

After several methods were explored, global deprotection of 11 in the presence of the azide group was eventually achieved in one step by acetylation\(^\text{17}\) using a mixture of sulphuric acid, acetic anhydride and acetic acid to afford the 4-deoxy-4-azido-myoinositol pentacetaate 4a\(^\text{18}\) in 65% yield. Minor amounts of the partially acetylated product 3-benzyl-6-azido-myoinositol tetracetaate 13 was also obtained, which could be transformed under the same conditions to yield 4a. Further methanolysis in the presence of a catalytic amount of sodium methoxide afforded the 4-deoxy-4-azido-myoinositol analogue 4b. Single crystals of pentacetaate 4a, from 1:1 hexane/ethyl acetate, confirmed the stereochimsttry unambiguously by X-ray analysis (Figure 2).

**Figure 2. X-ray structure of racemic pentacetaate 4-azide analogue 4a.**

Synthesis of meso-5-Deoxy-5-Azido myo-Inositol 5:

Starting with the dicyclohexylidene diol 6, benzylisation of the two remaining hydroxyl groups afforded the fully protected inositol derivative 14 in excellent yield (Scheme 2). The kinetically labile and slightly distorted trans-ketal of the compound 14 was cleaved selectively by controlled acid hydrolysis using acetyl chloride in DCM/MeOH (3:1) to afford the diol 15 in 68% yield.\(^\text{19}\) The diol 15 was regioselectively benzylated under phase transfer catalyst (tetrabutyl ammonium hydrogen sulphate) conditions, resulting in a separable mixture of benzylated products 16 and 17.\(^\text{20}\) Due to difficulties to distinguish 16 from 17, both alcohols were converted to their corresponding methylated derivatives 18a,b for identification purposes. Subsequent removal of the cis-ketal unit and global benzylation of 18a,b provided the fully protected inositol derivatives 20a and 20b, respectively. The \(^1\)H and \(^13\)C NMR of the derived meso-compound (20b) clearly indicated the structure to be 5-O-methyl-1,2,3,4,6-penta-O-benzyl myo-inositol. Thus, the corresponding starting alcohol 17 was confirmed to possess the C\(_6\)-free alcohol for the synthesis of the desired 5-series azido-analogues 5.

**Figure 2. X-ray structure of meso pentacetaate 5-azido analogue 5a.**
regenerate the myo-configured product 24 in good yield. Interestingly, no elimination (enol) product was detected, presumably due to 1,3-diaxial steric preventing E2-elimination. Compound 24 was then subjected to exhaustive acetylation to form the 5-deoxy-5-azo-myo-inositol pentaacetate 5a. In this case, an elevated temperature 60 °C was required for complete conversion to the pentaacetate 5a. The isomeric, partially acetylated, benzyl ether products were also converted to 5a under the same acetylation conditions. Deacetylation of 5a was accomplished by methanolysis in the presence of catalytic amounts of sodium methoxide to yield 5-deoxy-5-azo-myo-inositol 5b. Single crystals of compound 5a from 1:1 hexane/ether confirmed the structure unambiguously by X-ray analysis (Figure 3).

![Figure 3](image-url)  
**Figure 3.** X-ray structure of meso pentaacetate 5-azo analogue 5a.

**Synthesis of (±)-3-Deoxy-3-Azido myo-Inositols 3:**

Inspired by the report of Watanabe et al. for an efficient S₉,2 substitution of C₃-inositol triflates, the synthesis of the 3-series 3a,b was studied by consecutive double substitution at the C₅ position of an inositol intermediate 26, which was obtained from the previously synthesized inositol derivative 7 by subsequent methoxy methyl (MOM) ether protection to form the fully protected 25 and Pd-C hydrogenolysis (Scheme 3). The C₅-free alcohol 26 was treated with trifluoromethane sulfonylic acid anhydride in pyridine to yield the corresponding triflate 27, which was immediately reacted with potassium acetate to form the triflate 28 in 90% yield without elimination, presumably due to the less basic nature of the acyl anion as compared to azide species.

Deacetylation of 28 in the presence of catalytic amounts of sodium methoxide gave the axial alcohol 29 (Scheme 3). After completion, acidic resin was added to the reaction mixture to remove sodium ions by ion exchange. Unexpectedly, the trans ketal unit of 29 rearranged into a more stable cis C₃/C₄-ketal (30) by ketal migration under the slightly acidic conditions. Hence, a basic aqueous work up procedure using ethylacetate-water was employed while scaling up. The free axial alcohol in 29 was subsequently treated with triflic anhydride and pyridine to form the triflate 31. Excess pyridine led to formation of an eliminated product (35) in minor amounts. Two equivalents of pyridine in dichloromethane, however, generated the trifluoromethane sulfonylated product 31 in 90% yield without elimination. On the other hand, the free axial alcohol of 29 could be smoothly mesylated in pyridine as the solvent to form 32 without elimination, presumably due to the lower leaving group aptitude of OMs as compared to OTf. The isolated triflate 31 was examined first. Treatment with sodium azide in DMF generated the myo-configured substitution product 33 in low yield (15%) together with a minor S₅,1 substitution product 34 (10%) and the elimination product 35 in 56% yield. In comparison, the mesylate derivative 32 failed to undergo azide displacement with sodium azide in DMF, even upon heating at 70 °C. Also, Mitsunobu reaction of the alcohol 29 in order to achieve direct azide displacement was unsuccessful.

**Scheme 3.** Synthesis of (±)-3-azo myo-inositol analogues 3a,b. Reagents and conditions: i. MOMCl, DIPEA, 0 °C to rt, 87%; ii. EtOAc, THF, 20% Pd-C, H₂, 60%; iii. TiO₂, Py, DCM, 0 °C, 78%; iv. KOAc, DMA, 70 °C, 96%; v. NaOMe, MeOH, rt, 97%; vi. (iii), (ii); vii. MeSO₂Cl, py, 94%; viii. Na₂NN/Bu₄NF/THF.

Eventually, the combination of excess TMSN₃ (8 equiv.) in the presence of (0.5 equiv.) of TBAF in THF medium provided myo-azide 33 in a 43% optimal yield. To complete the 3-series, global acetolysis of compound 33 formed the desired 3-deoxy-3-azo myo-inositol pentaacetate 3a in moderate yield (Scheme 3) under mildly acidic conditions (2% HSO₄ in acetic anhydride). Treatment of 33 in dry, ethereal HCl further allowed all acyl labile protecting groups to be cleaved in one step and 3-deoxy-3-azo myo-inositol 3b could be isolated cleanly as a white solid after an ether wash.

**Synthesis of meso-2-Deoxy-2-Azido myo-Inositols 2:**

Following the orthoformate protection approach of Kishi et al., which allows for large differences in reactivity between equatorial and axial hydroxyl groups of inositol in terms of steric and electronics, a route to the 2-series of azide analogues was developed (Scheme 4). The symmetric dibenzy myo-inositol orthoformate 37 was prepared from the dibenzoate derivative 36 through a prolonged silver(I) oxide chelation mediated bis-benzylation and anamolysis sequence. The equatorial free hydroxyl group of 37 was oxidised under DMF condition to give the keto 38 in excellent yield. Next, stereoselective reduction of ketone 38 produced the inverted axial alcohol 39 in the scyllo-configuration exclusively. The rigidity of the orthoformate unit presumably accounts for this highly stereoselective reduction step. The axially positioned free alcohol of 39 was then sulfonylated by treatment with methane sulfonf chloride in pyridine to give 40 in excellent yield. At this stage, an S₂,2 attack at C₅ of compound 40 was presumed to be sterically challenging. Hence, orthoformate cleavage of compound 40 was performed first, and the triol 41 was formed by mild methanolysis with pTSA. Next, the mesylate derivative 41 was heated with sodium azide in DMF, which formed the myo-configured azide substituted product 42 in moderate yield, along with minor amounts of the epoxide 43 and some unreacted starting material 41. No elimination product was identified in this case; however, the reaction required heating at 80 °C, which led to decomposition of some material. Final exhaustive acetylation of 42 completed the synthesis of the desired 2-deoxy-2-azo myo-inositol pentaacetate 2a in good yield. Clean methanolation under
basic conditions regenerated the free hydroxyl groups to produce meso-2-deoxy-2-azido-myo-inositol 2b.

![Scheme 4. Synthesis of 2-azido myo-inositol analogues 2a,b. Reagents and conditions: i. (a) Ag_2O, BuBr, DMF, rt, (b) (CH_3)_2CHCHNH_2, MeOH, reflux, 70%; ii. DMP, DCM, rt, 95%; iii. MeOH, THF, NaBH_4, 87%; iv. CH_3SOCl, py, 92%; v. pTSA, MeOH, 97%; vi. NaN_3, DMF, 80 °C; vii. 42, Ac_2O, AcOH, 15% H_2SO_4 in Ac_O, 50 °C, 64%; viii. NaOMe, MeOH, 99%.](image)

**Summary**

In this letter, we have described straightforward routes to various azido-deoxy inositol analogues through azide installation. As a common strategy, we adopted a consecutive S_2 double inversion approach on suitably protected inositol derivatives, which were derived via convenient oxidation-reduction sequences from myo-inositol. A final, global acetylation step in the presence of the azide group (2-15% sulphuric acid in acetic anhydride/acid) enabled the direct and convenient synthesis of azido-inositol pentacacetate analogues 2a-5a. Methanolation by treatment with catalytic sodium methoxide subsequently provided the fully unprotected azido myo-inositol surrogates 2b-5b cleanly. The routes are convenient and provide sufficient material for biological study. Having genuine azido surrogates of myo-inositol in hand, a more concise and diversified strategy is under investigation. In the meantime, the metabolic incorporation of these modified azido inositol analogues into various inositol lipids of yeast cells are in progress, and lipid profiling and biosynthetic cell compatibilities will be reported in due course.

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**Supplementary data**

Supplementary data associated with this article can be found, in the online version, at …

**References and notes**

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