Allosteric Optical Control of a Class B G-Protein-Coupled Receptor

Johannes Broichhagen, Natalie R. Johnston, Yorrick von Ohlen, Helena Meyer-Berg,
Ben J. Jones, Stephen R. Bloom, Guy A. Rutter, Dirk Trauner,* and David J. Hodson*

Abstract: Allosteric regulation promises to open up new therapeutic avenues by increasing drug specificity at G-protein-coupled receptors (GPCRs). However, drug discovery efforts are at present hampered by an inability to precisely control the allosteric site. Herein, we describe the design, synthesis, and testing of PhotoETP, a light-activated positive allosteric modulator of the glucagon-like peptide-1 receptor (GLP-1R), a class B GPCR involved in the maintenance of glucose homeostasis in humans. PhotoETP potentiates Ca^{2+}, cAMP, and insulin responses to glucagon-like peptide-1 and its metabolites following illumination of cells with blue light. PhotoETP thus provides a blueprint for the production of small-molecule class B GPCR allosteric photoswitches, and may represent a useful tool for understanding positive cooperativity at the GLP-1R.

The incretin hormone glucagon-like peptide-1 (GLP-1) is released from enteroendocrine L-cells in the intestine,[1] from where it binds cognate receptors to promote the survival of pancreatic beta cells, insulin release, and weight loss.[2] For these reasons, incretin mimetics based on GLP-1 have become widely-prescribed drugs for the restoration of normal glucose levels in type 2 diabetes (T2D),[3] a socio-economically costly syndrome affecting almost 400 million individuals worldwide.[4]

The glucagon-like peptide 1 receptor (GLP-1R) is a class B G-protein-coupled receptor (GPCR) that is primarily coupled to adenylate cyclase activity and 3′-5′-cyclic adenosine monophosphate (cAMP) accumulation,[5] as well as intracellular Ca^{2+} fluxes.[6] Recently, an allosteric site has been described for this receptor that allows fine modulation of orthosteric ligand binding.[7] The ligand-dependent allosteric activator 4-(3-(benzyloxy)phenyl)-2-(ethylsulfinyl)-6-(trifluoromethyl)pyrimidine (BETP) potentiates Ca^{2+} mobilization in response to GLP-1(7-36)NH_2,[7,8] the active amidated form of GLP-1. By contrast, BETP amplifies cAMP generation in response to GLP-1(9-36)NH_2,[7,8] a metabolite and weak partial GLP-1R agonist. Such interactions are therapeutically desirable, since drugs that target the GLP-1R allosteric site may improve receptor specificity, thereby reducing side effects.[7–9] However, their investigation is at present hindered by a lack of specific research tools for the fine control of allosterism and receptor movement. Photo-pharmacology is well-suited to this task, since it relies on the properties of light to precisely deliver drug activity in space and time.[10]

Herein, we describe the development and testing of PhotoETP, a light-activated positive allosteric modulator that allows optical control of GLP-1R signaling and insulin secretion by using blue light (Figure 1A).

We set out to confer photoswitching on the GLP-1R allosteric site by subjecting BETP to our "azologization" strategy[11] (Figure 1B; see also Figure S1 in the Supporting Information). By coupling commercially available chloropyrimidine **1** and boronic acid **2** under Suzuki–Miyaura conditions, bisaryl thioether **3** was obtained in a yield of 95%. After oxidizing the sulfur atom with *m*CPBA to its sulfone counterpart **4** in a yield of 90%, it was exchanged in an aromatic substitution with ethyl sulfide to give ethyl thioether **5** in a yield of 55%. Subsequent oxidation with one equivalent of *m*CPBA gave access to sulfoxide **6** (96%), which was deprotected with TFA before undergoing Mills reaction with

[*] Dr. J. Broichhagen, H. Meyer-Berg, Prof. Dr. D. Trauner
LMU Munich, Department of Chemistry and
Center for Integrated Protein Science (CIPSM)
Butenandtstrasse 5–13, 81377 Munich (Germany)
E-mail: dirk.trauner@lmu.de

Dr. J. Broichhagen
École Polytechnique Fédérale de Lausanne (EPFL)
Institute of Chemical Sciences and Engineering (ISIC)
Laboratory of Protein Engineering (LIP), 1015 Lausanne (Switzer-land)

N. R. Johnston, Y. von Ohlen, Prof. Dr. G. A. Rutter, Dr. D. J. Hodson
Imperial College London
Section of Cell Biology and Functional Genomics
Division of Diabetes, Endocrinology and Metabolism
Department of Medicine, Hammersmith Hospital
Du Cane Road, London W12 0NN (UK)
E-mail: d.hodson@imperial.ac.uk

Dr. B. J. Jones, Prof. Dr. S. R. Bloom
Imperial College London, Section of Investigative Medicine
Division of Diabetes, Endocrinology and Metabolism (UK)

Dr. D. J. Hodson
Institute of Metabolism and Systems Research (IMSR)
University of Birmingham, B15 2TT (UK)
and
Centre for Endocrinology, Diabetes and Metabolism
Birmingham Health Partners, Birmingham, B15 2TH (UK)
E-mail: d.hodson@bham.ac.uk

Supporting information (experimental details including synthesis, spectroscopic and spectrometric characterization, and biology) and the ORCID identification number(s) for the author(s) of this article can be found under http://dx.doi.org/10.1002/anie.201600957.
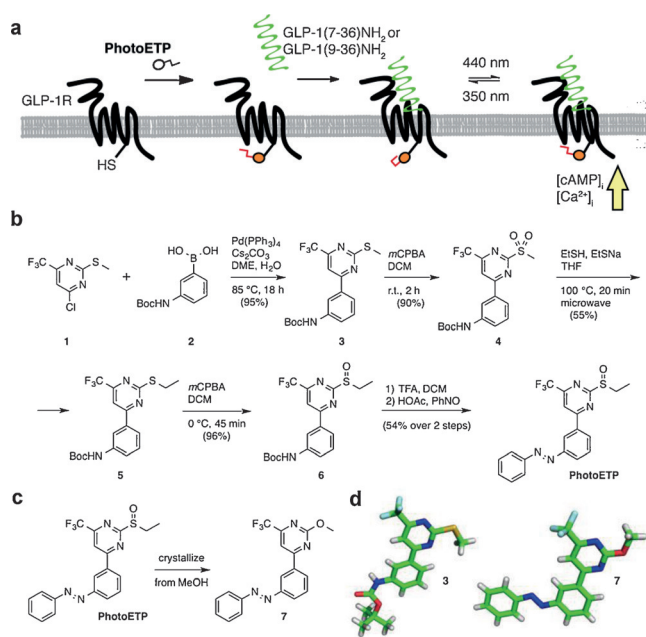
Figure 1. Design and synthesis of PhotoETP. a) An azobenzene unit is installed on the positive allosteric modulator BETP to produce Photo-ETP. This allows Ca^{2+} and cAMP responses to GLP-1 and its inactive metabolites to be potentiated following illumination with UV or blue light. b) Six-step synthetic pathway for the production of PhotoETP. c) Crystallization of PhotoETP as its methoxy counterpart (**7**) from MeOH. d) Crystal structures for PhotoETP congener **7** (CCDC 1420305 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre) and its precursor bisaryl thioether **3** (CCDC 1420306 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre).

Figure 2. Characterization of PhotoETP. a) Isomerization of PhotoETP between its *trans-* and *cis-* states with blue light or UV irradiation, respectively. b) UV/Vis spectra of PhotoETP in DMSO following illumination at $\lambda = 440$ nm (blue), $\lambda = 330$ nm (grey), or under dark conditions (black). c) Robust photoswitching between *trans-* and *cis-*PhotoETP induced with $\lambda = 440$ nm and $\lambda = 330$ nm, respectively. d) LC–MS trace of PhotoETP in the dark (black) and after exposure to UV light ($\lambda = 350$ nm; gray).

nitrosobenzene to produce PhotoETP in a yield of 54% over two steps. Attempts to crystallize PhotoETP from MeOH yielded compound **7**, thus providing further evidence for the electrophilicity of PhotoETP (Figure 1 C). Crystals suitable for X-ray crystallography were obtained for both **3** and **7** (Figure 1 D).

The UV/Vis spectrum of PhotoETP under illumination at $\lambda = 440$ nm (*trans*, blue) and $\lambda = 330$ nm (*cis*, gray) demonstrated the presence of wavelength-dependent switching (Figure 2 A), as expected for a *meta*-azobenzene system (Figure 2 B). Photoswitching could be repeated over several cycles with reasonably fast kinetics ($\tau_{cis} = 204.2 \pm 7.3$ s; $\tau_{trans} = 54.5 \pm 2.9$ s) and without obvious fatigue (Figure 2 C), and the *cis* and *trans* isomers were separated by LC–MS analysis (Figure 2 D). Together, these features of PhotoETP provide a basis for the allosteric photocontrol of GLP-1R activity.

We next examined whether PhotoETP was able to yield optical control over GLP-1(9-36)NH_2-induced cAMP generation. Similarly to BETP, *trans*-PhotoETP potentiated cAMP rises in response to GLP-1(9-36)NH_2 [EC$_{50}$(*trans*-Photo-ETP) = 163.3 nm; EC$_{50}$(BETP) = 99.5 nm]. Importantly, this activity could be switched off using UV illumination to induce *cis*-isomer formation (Figure 3 A; unable to calculate EC$_{50}$). The extent of photoswitching is similar to that recently reported for an allosteric modulator of the metabotropic
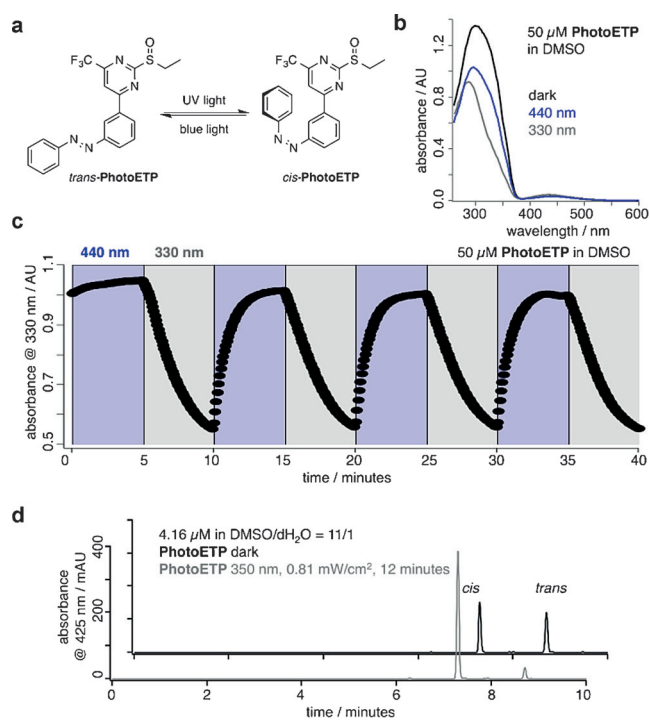
glutamate receptor mGluR5, a class C GPCR.[12] The effect of PhotoETP on cell viability was determined in islets by using necrosis and apoptosis assays in the dark. At the concentration used throughout the present study, PhotoETP did not induce significant necrosis (Figure 3 B) or apoptosis (Figure 3 C), as measured using propidium iodide incorporation and terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL), respectively. By contrast, treatment with staurosporine or thapsigargin positive controls resulted in large increases in necrotic and apoptotic indices (Figure 3 B,C). Furthermore, levels of cleaved caspase-3, an enzyme involved in the proteolytic cleavage of critical intracellular effectors including poly (ADP-ribose) polymerase, were unaffected by incubation with PhotoETP (Figure S2).

Next, photoswitching of intracellular Ca^{2+} dynamics was assessed by using PhotoETP directly in beta cells residing within intact islets of Langerhans. Whereas *cis*-PhotoETP ($\lambda = 350$ nm) showed little effect, the *trans* isomer ($\lambda = 440$ nm) potentiated GLP-1(7-36)NH_2-induced increases in Ca^{2+} levels (Figure 3 D–G), as previously described for cAMP. The latter could be abolished by using either low glucose (Figure S3A,B) or the specific GLP-1R antagonist exendin 9–39 (Figure S3C,D). In all cases, results in islets were replicated in MIN6 beta cells subjected to high-throughput Ca^{2+} screens (Figure 3 H–J). When using batch-incubated islets, *trans*-PhotoETP potently amplified GLP-1(9-36)NH_2-induced insu-
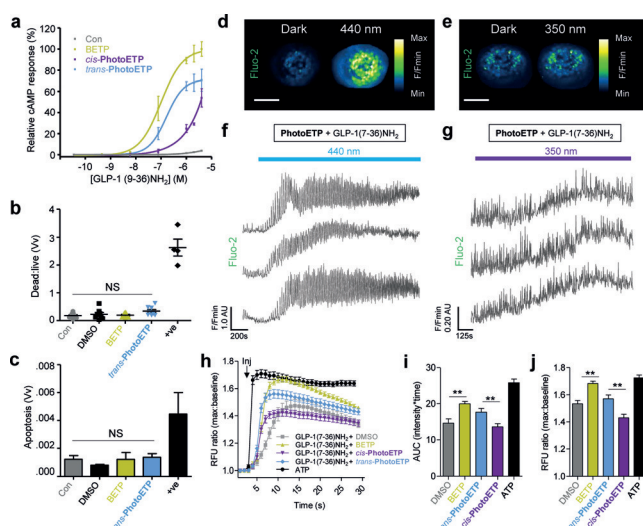
Figure 3. Optical control of cAMP and Ca²⁺ signals. a) Illumination at
λ = 440 nm, but not λ = 350 nm (UV), optically modulates GLP-1(9-
36)NH₂-induced cAMP generation in CHO-GLP-1R cells (n = 4
repeats). b) Incubation of MIN6 beta cells with PhotoETP for 3 h does
not alter cell viability (n = 8 repeats, +ve = positive control: stauro-
sporine or thapsigargin). c) As for (b) but with apoptosis measured
(n = 3 repeats). d–g) Illumination at λ = 440 nm, but not λ = 350 nm,
modulates GLP-1(7-36)NH₂-induced Ca²⁺ increases in intact Photo-
ETP-treated islets (n = 9 recordings from 3 animals). h–j) As for (d)–
(g), but in MIN6 beta cells (n = 8 repeats; RFU = relative fluorescence
units, AUC = area under the curve; ATP 10 μM; positive control). In all
cases, GLP-1(7-36)NH₂ was co-applied at 10 nM in the presence of 8–
11 mM (islets) or 17 mM (MIN6) D-glucose. PhotoETP and BETP were
applied at 50 μM. NS: non-significant or **P < 0.01 versus cis-Photo-
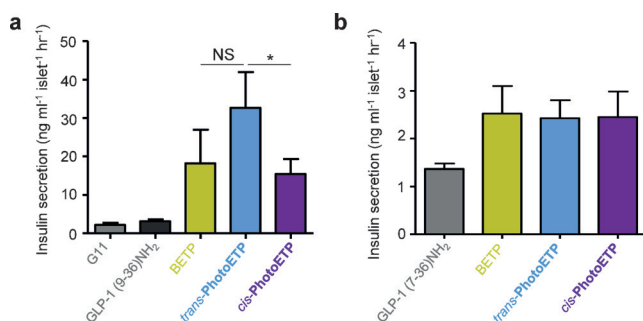ETP or DMSO; one-way ANOVA. Values represent the mean ± SEM.
Scale bar, 75 μm.

Figure 4. Optical control of insulin secretion. a) trans-PhotoETP is
more effective than cis-PhotoETP at potentiating GLP-1(9-36)NH₂-
induced insulin secretion. b) trans-PhotoETP and cis-PhotoETP similarly
potentiate GLP-1(7-36)NH₂-induced insulin secretion. In all cases,
PhotoETP and BETP were applied at 50 μM with either GLP-1(9-
36)NH₂ 100 nM or GLP-1(7-36)NH₂ 10 nM, as indicated. D-glucose
was present at 11 mM. NS: non-significant or *P < 0.05 versus cis-
PhotoETP or BETP; one-way ANOVA. Values represent the mean ±
SEM.

lin secretion (Figure 4A), whereas cis-PhotoETP was less
effective as an allosteric modulator (Figure 4A). By contrast,
BETP, cis-PhotoETP, and trans-PhotoETP all augmented
insulin secretion in response to GLP-1(7-36)NH₂ by almost 2-
fold (Figure 4B), with no evidence of photocontrol.

PhotoETP was almost 20% cis-enriched under benchtop
conditions (¹H NMR spectrum; see the Supporting Informa-
tion), whereas the UV/Vis spectra revealed a more pro-
nounced π–π* band in the dark (see Figure 2B). This finding
can be explained by consulting a model of the glucagon
receptor mutant F345C bound to BETP, where a twisted
conformation of the benzylether is found to be the lowest
energy state.[7] Such a conformation may also be adopted by
cis-PhotoETP owing to its higher affinity for covalent binding.
However, in contrast to BETP, which can reorganize its
molecular shape in response to orthosteric ligand binding,
PhotoETP would remain trapped in its cis state until
illumination to induce trans-isomerization. Although the
exact isomer ratio at the receptor is difficult to determine
empirically, such properties may nonetheless afford fine
control over photoswitching, with dark conditions, 440 nm
illumination, and 350 nm illumination leading to graded Ca²⁺
responses (Figure S4). Further studies using rigid E- and Z-
stilbene bioisosteres of PhotoETP will be required to better
delineate the mechanisms involved.

The data presented herein outline a straightforward
synthetic strategy for the production of a blue-light-activated
positive allosteric modulator, which enables photocontrol of
GLP-1R activity through a feedforward loop encompassing
the orthosteric site (Figure 1A). Although similar "allos-
witches" have been described for ionotropic and metabo-
tropic mGluRs,[12,13] this is the first demonstration of their use
in a therapeutically relevant class B GPCR. Using a combi-
nation of Ca²⁺, cAMP, and insulin assays in CHO-GLP-1R
and MIN6 cells, as well as islets of Langerhans, we were able
to show that PhotoETP allows photoswitching of responses to
GLP-1(7-36)NH₂ and its less active breakdown product,
GLP-1(9-36)NH₂, with similar potency to native BETP.
Notably, PhotoETP displays unusual behavior in cells,
where it shows an enriched cis content when interacting
with its target in the dark. Indeed, the more active trans
isomer has to be photochemically induced by irradiation with
blue light. As a result of these properties, PhotoETP, together
with the recently described signal-biased GLP-1R photo-
switch LirAzo,[14] may enable the precise dissection of
allosteric–orthosteric cooperativity, molecular movement,
and binding.

Both BETP and PhotoETP were more effective at
potentiating GLP-1(9-36)NH₂-induced compared to GLP-
1(7-36)NH₂-induced insulin secretion. This suggests that
cAMP rather than Ca²⁺ is the primary driver of the "incretin
effect", and is consistent with previous results obtained using
LirAzo.[14] Intriguingly, optical control of insulin release could
only be observed in GLP-1(9-36)NH₂- and PhotoETP-treated
islets, where blue light provoked a two-fold higher response
than UV illumination. Although the exact reasons for this
remain unknown, it may reflect an inability to detect
relatively small isomer-induced differences in intracellular
Ca²⁺ versus cAMP concentration at the level of secretion in
islets.

BETP was susceptible to UV-A-induced but not white-
light-induced reactions, thus making it a poor control for
photoswitching purposes (see Figure S5–S8). In contrast,
PhotoETP was remarkably robust. This protective effect

stems from the azobenzene unit, which preferentially harvests UV-A photons with its π–π^* band to undergo isomerization. In other words, by installing an azobenzene moiety onto BETP, side reactions can be quenched and the resulting molecule stabilized. Nevertheless, the UV-A-induced rearrangement of BETP to its sulfenic ester counterpart via a Meisenheimer complex, and the accompanying transformation, is in itself an interesting finding (Figure S5,S6). Although related rearrangements of sulfoxides have been reported,[15] sulfenic esters have not been isolated as products owing to the low (UV-C) wavelengths used in these experiments. Such rearrangements are relevant for drug activity, as best exemplified by acid-activation of the irreversible proton-pump inhibitor omeprazole (Prilosec).[16]

In summary, we showcase PhotoETP, a light-activated modulator for allosteric optical control of GLP-1R function, and highlight the requirement to run parallel control experiments with benchmark drugs in photopharmacology. Photo-ETP, or optimized derivatives thereof, may be useful in drug-discovery programs aimed at unraveling the complexity of allosterism and class B GPCR signaling.

Acknowledgments

Keywords: allosteric regulation · beta cells · GLP-1 receptor · photopharmacology · type 2 diabetes

[1] H. E. Parker, F. Reimann, F. M. Gribble, *Expert Rev. Mol. Med.* **2010**, *12*, e1.
[2] J. E. Campbell, D. J. Drucker, *Cell Metab.* **2013**, *17*, 819–837.
[3] S. E. Inzucchi, R. M. Bergenstal, J. B. Buse, M. Diamant, E. Ferrannini, M. Nauck, A. L. Peters, A. Tsapas, R. Wender, D. R. Matthews, *Diabetes Care* **2012**, *35*, 1364–1379.
[4] C. J. Nolan, P. Damm, M. Prentki, *The Lancet* **2011**, *378*, 169–181.
[5] a) D. Gefel, G. K. Hendrick, S. Mojsov, J. Habener, G. C. Weir, *Endocrinology* **1990**, *126*, 2164–2168; b) B. Thorens, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 8641–8645; c) G. G. Holz IV, C. A. Leech, J. F. Habener, *J. Biol. Chem.* **1995**, *270*, 17749–17757.
[6] a) J. Gromada, K. Bokvist, W. G. Ding, J. J. Holst, J. H. Nielsen, P. Rorsman, *Diabetes* **1998**, *47*, 57–65; b) G. G. Holz, C. A. Leech, R. S. Heller, M. Castonguay, J. F. Habener, *J. Biol. Chem.* **1999**, *274*, 14147–14156; c) G. G. Holz, *Diabetes* **2004**, *53*, 5–13.
[7] W. M. Nolte, J. P. Fortin, B. D. Stevens, G. E. Aspnes, D. A. Griffith, L. R. Hoth, R. B. Ruggeri, A. M. Mathiowetz, C. Limberakis, D. Hepworth, P. A. Carpino, *Nat. Chem. Biol.* **2014**, *10*, 629–631.
[8] D. Wootten, E. E. Savage, C. Valant, L. T. May, K. W. Sloop, J. Ficorilli, A. D. Showalter, F. S. Willard, A. Christopoulos, P. M. Sexton, *Mol. Pharmacol.* **2012**, *82*, 281–290.
[9] F. S. Willard, D. Wootten, A. D. Showalter, E. E. Savage, J. Ficorilli, T. B. Farb, K. Bokvist, J. Alsina-Fernandez, S. G. Furness, A. Christopoulos, P. M. Sexton, K. W. Sloop, *Mol. Pharmacol.* **2012**, *82*, 1066–1073.
[10] a) W. A. Velema, W. Szymanski, B. L. Feringa, *J. Am. Chem. Soc.* **2014**, *136*, 2178–2191; b) J. Broichhagen, M. Schönberger, S. C. Cork, J. A. Frank, P. Marchetti, M. Bugliani, A. M. J. Shapiro, S. Trapp, G. A. Rutter, D. J. Hodson, D. Trauner, *Nat. Commun.* **2014**, *5*, 5116; c) J. Broichhagen, J. A. Frank, N. R. Johnston, R. K. Mitchell, K. Šmid, P. Marchetti, M. Bugliani, G. A. Rutter, D. Trauner, D. J. Hodson, *Chem. Commun.* **2015**, *51*, 6018–6021.
[11] J. Broichhagen, J. A. Frank, D. Trauner, *Acc. Chem. Res.* **2015**, *48*, 1947–1960.
[12] S. Pittolo, X. Gomez-Santacana, K. Eckelt, X. Rovira, J. Dalton, C. Goudet, J. P. Pin, A. Llobet, J. Giraldo, A. Llebaria, P. Gorostiza, *Nat. Chem. Biol.* **2014**, *10*, 813–815.
[13] M. Volgraf, P. Gorostiza, R. Numano, R. H. Kramer, E. Y. Isacoff, D. Trauner, *Nat. Chem. Biol.* **2006**, *2*, 47–52.
[14] J. Broichhagen, T. Podewin, H. Meyer-Berg, Y. von Ohlen, N. R. Johnston, B. J. Jones, S. R. Bloom, G. A. Rutter, A. Hoffmann-Röder, D. J. Hodson, D. Trauner, *Angew. Chem. Int. Ed.* **2015**, *54*, 15565–15569.
[15] a) M. Chen, R. Yang, R. Ma, M. Zhou, *J. Phys. Chem. A* **2008**, *112*, 7157–7161; b) Y. Guo, Iowa State University **1997**.
[16] W. O. Foye, T. L. Lemke, D. A. Williams, *Foye's principles of medicinal chemistry*, 6th ed., Lippincott Williams & Wilkins, Philadelphia, **2008**.